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VOLUME 36 JUNE 1958 NUMBER 3

RELATIONSHIP OF RESPIRATION RATE TO TEMPERATURE IN A SUPERCOOLED INSECT[1]

R. W. SALT[2]

Abstract

The rate of oxygen consumption of mature larvae of *Anagasta kühniella* (Zell.) exhibits a logarithmic relationship to temperature over a range extending from the limit of supercooling to the beginning of heat injury. This information supplements that of Scholander *et al.* (4), who demonstrated the same relationship in frozen *Chironomus* larvae, the rate being lower than in supercooled forms and hence not continuous with the above-zero portion of the curve. Together, these two simple relationships invalidate Kozhantschikov's theory of cold-hardiness based on a thermostable respiration below 0° C. in cold-hardy insects and no respiration in frozen non-cold-hardy insects.

Introduction

In 1938 Kozhantschikov (1) drew analogies between the cold-hardiness of some insects and their respiratory rates below 0° C. His results indicated that several hibernating, diapausing, cold-hardy forms maintained a fairly high level of respiration below 0° C., which he termed thermostable. In contrast, certain non-diapause, non-cold-hardy forms exhibited no measurable respiration below their freezing points, which lay not far below 0° C. Kozhantschikov's methods have been challenged by Scholander *et al.* (4), who point out that, when his material was heated to 0° C. or higher to make a reading, two periods of highly accelerated respiration were included in the measurements. Further criticisms may be made: the design of the respirometer and some of the measurements imply insufficient sensitivity at low respiratory levels; the term *cold-hardy* is used loosely, in some cases meaning ability to survive freezing and in others ability to avoid freezing by supercooling; respiratory rates below 0° C. are treated as though the state of the insect in respect to cold-hardiness and diapause is paramount and the frozen or supercooled state of little importance.

Scholander *et al.* (4) measured the oxygen consumption of frozen *Chironomus* larvae down to −15° C. and found a direct logarithmic relationship to temperature below the freezing point (Fig. 1). Above 0° C., the slope of the curve is different; although the data in this range are sparse, it is likely, in

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view of results presented here, that another straight line relationship exists between 0° C. and about 15° C. The rate of increase slowly falls off above 15° C. and respiration is already declining at 30° C. as a result of heat injury. That this reversal occurs at such a low temperature reflects the physiological adaptation of these cold-hardy, freezing-resistant larvae that were chopped out of frozen Arctic mud.

The present experiments were designed to establish the temperature–oxygen consumption curve of a supercooled insect and to determine whether it forms an extension of the above-zero portion of the curve.

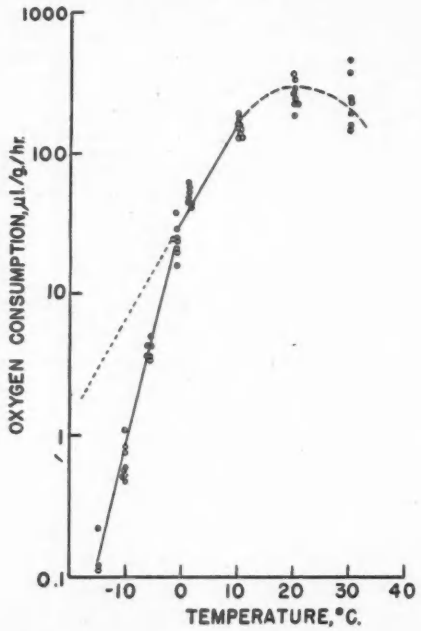

FIG. 1. Influence of temperature on oxygen consumption of *Chironomus* larvae chopped from frozen Arctic mud. Measurements below 0° C. made on frozen larvae. Solid lines fitted by eye; the broken line represents the probable relationship in supercooled larvae (cf. Fig. 2). Redrawn from Scholander, Flagg, Hock, and Irving (4).

Materials and Methods

The experimental insects were mature larvae of the Mediterranean flour moth, *Anagasta kühniella* (Zell.), taken from large cultures when they ceased feeding and began wandering in search of a place to spin a cocoon. Fifty larvae were tested individually at each temperature. At −15° C. most of the larvae froze, so prepupal larvae, which remained supercooled at this temperature, were substituted. However, since their respiration rate was lower than that of the mature larvae, measurements were made also at −10° and −5° C. to establish this segment of the respiration–temperature curve.

Oxygen consumption was measured periodically over periods of 6 to 8 hours in a Warburg apparatus at temperatures of 20° C. and higher, and in Kirk-Barth microrespirometers at temperatures of 15° C. and lower.

Results

The mean values of oxygen consumption are plotted logarithmically against temperature in Fig. 2. The slope of the curve does not change at the freezing point, as it does when the insects are frozen (cf. Fig. 1). The respiration rate is essentially constant from the limit of supercooling up to about 30° C., where it begins to show the effects of heat injury that become pronounced above 35° C. The short, lower curve extends the results to −15° C. and shows that the prepupae, with a lower respiration rate, exhibit the same relationship between oxygen consumption and temperature.

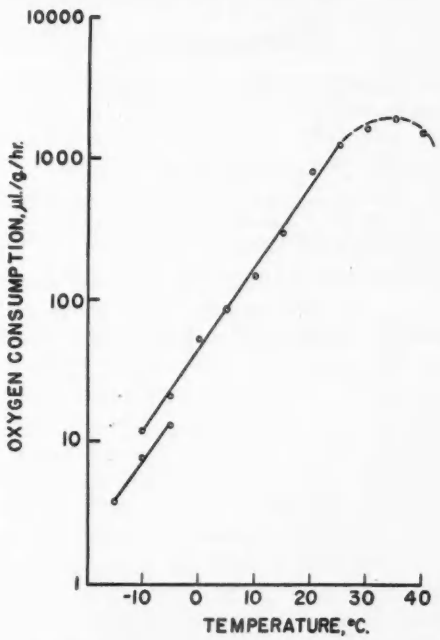

FIG. 2. Influence of temperature on oxygen consumption of larvae of *Anagasta kühniella* (Zell.). Upper curve, mature larvae before spinning; lower curve, prepupal larvae from cocoons. Solid lines fitted by method of least squares.

Discussion

It is apparent that the respiration rate of *A. kühniella* larvae varies logarithmically with temperature between the limit of supercooling and the point where heat injury commences. In frozen *Chironomus* larvae the logarithmic relationship to temperature is maintained, but at a greatly reduced rate (4).

In view of the simple, clear-cut nature of these findings, and the objections to Kozhantschikov's work already mentioned, his theory of cold-hardiness based on the supposed existence of a thermostable subzero respiration is untenable. Since the appearance of Kozhantschikov's work in 1938 the endocrine control of diapause and the accompanying pattern of respiration have been elucidated (2). In addition, the relationship of diapause to cold-hardiness has been shown to be secondary, since diapausing forms are cold-hardy because they do not feed (3). Any influence of cold-hardiness on respiration is therefore indirect. In the present work the experimental insect does not undergo diapause and belongs to a species that cannot be considered cold-hardy even though some of the non-feeding stages supercool to about −25° C. Kozhantschikov's use of the term *cold-hardy* was very loose, referring mostly to insects able to survive some freezing but sometimes to freezing-susceptible species able to supercool 20° C. or more.

Acknowledgment

The able technical assistance of Mr. A. G. Hewitt of the Lethbridge laboratory is gratefully acknowledged.

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MONHYSTERA CAMERONI N. SP.—A NEMATODE COMMENSAL OF VARIOUS CRUSTACEANS OF THE MAGDALEN ISLANDS AND BAY OF CHALEUR (GULF OF ST. LAWRENCE)[1]

G. STEINER

Abstract

The known cases of monhysterid nematodes showing commensalism with crustaceans are reviewed and a new one, that of *Monhystera cameroni* n. sp., described. Its relationship to *M. socialis* Bütschli, 1874 and *M. chitwoodi* n. sp. (= *M. socialis* Bütschli of Chitwood, 1951) is discussed. *M. chitwoodi* is established as a separate species. It is proposed to replace the term "caudal" glands by that of "foot" glands for reasons of clarity and comparative morphology. The reduction of the foot gland system from a three to a two cell complex as here observed is interpreted. Its possible significance for the concept that the phasmids are modified foot glands is expounded. The occurrence of microsporidial parasites in *M. cameroni* is mentioned.

Introduction

In 1915 Baylis (6) first described two monhysterid nematodes, *Monhystera wilsoni* and *M. carcinicola*, as commensals of crustaceans; both were found living in the gill chambers of the land crabs, *Gecarcinus ruricola* (Linn.) and *Cardisoma guanhumi* Latreille, in Jamaica. Cobb (10) reassigned both these nematodes to new genera: *Monhystrium* and *Tripylium*. In the former genus he placed as type *M. transitans*, also a species from *Gecarcinus ruricola* and *M. wilsoni* (Baylis); in the latter, *T. carcinicolum* (Baylis). Chitwood later (9) assigned the genus *Monhystrium* to the Linhomoeïnae Filipjev, 1929 and *Tripylium* to the Sphaerolaiminae Filipjev, 1929.

In Germany the occurrence of a rich association of Ciliata, Oligochaeta, Rotatoria, Harpacticidae, Halacarina, and Nematoda in the gill chambers of *Potamobius* and *Cambarus* had been ascertained during the 1920's. Schneider (17) thus listed 12 species of nematodes representing groups with different feeding habits; only one species of the Monhysteridae, *Monhystera dispar* Bastian, 1865, a rather common fresh-water form, was amongst them.

In 1933, Allen (1) described "*Rhabditis*" *cambari* from the gills of *Cambarus acuminatus* Faxon and *Cambarus blandingii* Harlan, two crayfish from North Carolina. However, Chitwood (8) demonstrated that this "*Rhabditis*" was actually a *Monhystera*. He redescribed the form as *M. cambari* (Allen, 1933) Chitwood, 1935. In the same paper he added a new subspecies to *Tripylium*, *T. carcinicolum calkinsi* from the gills of *Gecarcinus lateralis* (Freminville) collected at Boca de Cangrejos, Puerto Rico. In 1953, Kinne and Gerlach (14) reported on another new monhysterid commensal, *Gammarinema gammari* Kinne and Gerlach, from *Gammarus locusia* Say, *G. oceanicus* (Sexton) Segerstrole, *G. salinus* (Sexton) Spooner, and *G. duebeni* Lilljeborg, all occurring in the Baltic Sea at Kiel, Germany. Finally, in 1955, Osche (15) reported on the

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Contribution from the Agricultural Experiment Station at the University of Puerto Rico, Rio Piedras, Puerto Rico.

occurrence of *Monhystera disjuncta* Bastian, 1865, between the "pereiopoda" of the gammaride *Orchestia gammarellus* (Pallas) collected on the coast of the Adriatic Sea at Rovigno. In the same paper Osche also reassigned the above mentioned *Monhystera cambari* (Allen) Chitwood to *Gammarinema* as *G. cambari* (Allen, 1933) Osche, 1955.

These are the presently known instances of association and commensalism of monhysterid nematodes with crustaceans. The additional case which is reported here had been discovered by Miss Betty J. Myers of the Institute of Parasitology at Macdonald College while she was engaged in a search of possible intermediate hosts of *Porrocaecum decipiens* (Krabbe, 1878). During this search the nematode here studied had been encountered adhering to the maxillipeds and mandibles and in some cases it had been found even in the digestive tract of *Mysis stenolepsis* Smith, *Mysis mixta* Lilljeborg, *Crago septemspinosus* Say, *Pandulus borealis* Krüger, and *Pandulus montagui* Leach. The material had been collected at the Magdalen Islands and the Bay of Chaleur in the Gulf of the St. Lawrence in the summer of 1956. The nematode involved is tentatively placed in the genus *Monhystera*, pending a revision of the whole family of Monhysteridae. The taxonomy of this group is at present much confused; it is a large group of mainly marine, to a lesser degree freshwater, but only sparingly soil-inhabiting free-living nematodes. Some of them are known to feed on algae, particularly diatoms. Consequently the group is thought to be vegetarian. The commensalism here encountered could be interpreted as a turn to a carnivorous and eventually parasitic mode of life. However, this is not necessarily so since even a gill-chamber habitat would not exclude vegetarianism.

Description of the New Species

Body cylindrical, subfiliform, tapering uniformly from base of oesophagus to less than one-half at the head end; tail end of female uniformly conical from vulva; that of male, from anus to tail tip (Figs. 1, 2, 12, 13, and 14). Color brown, quite opaque, lighter at two distal foot gland cells, at excretory gland, and at two first cells of intestine (Figs. 12, 14, and 5). Cuticle smooth, not annulated, setae almost completely absent; lateral fields not differentiated but

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FIGS. 1 to 9. *Monhystera cameroni* n. sp.

FIG. 1. Male, left side up, *e.g.* excretory gland, located underneath, and farther back than in all other specimens seen; *p.g.* progaster. In order to demonstrate better the testis and its outlet, the intestine, which is on top, is only partly drawn. 280×

FIG. 2. Young female, right side up. 280×

FIG. 3. Contents of intestinal canal of male as seen about four times body width in front of anus. 315×

FIG. 4. Head end of a larva right ventral side turned up; amphids not quite in profile. 315×

FIG. 5. Anterior end of female demonstrating excretory gland; progaster and cardia. 315×

FIG. 6. Larval specimen exhibiting an infection with Sporozoa.

FIG. 7. Portion of larva of 0.440 mm. length to demonstrate the sexual anlage. 315×

FIG. 8. Sporozoa highly magnified. 1333×

FIG. 9. Youngest larva observed with sexual anlage. 315×

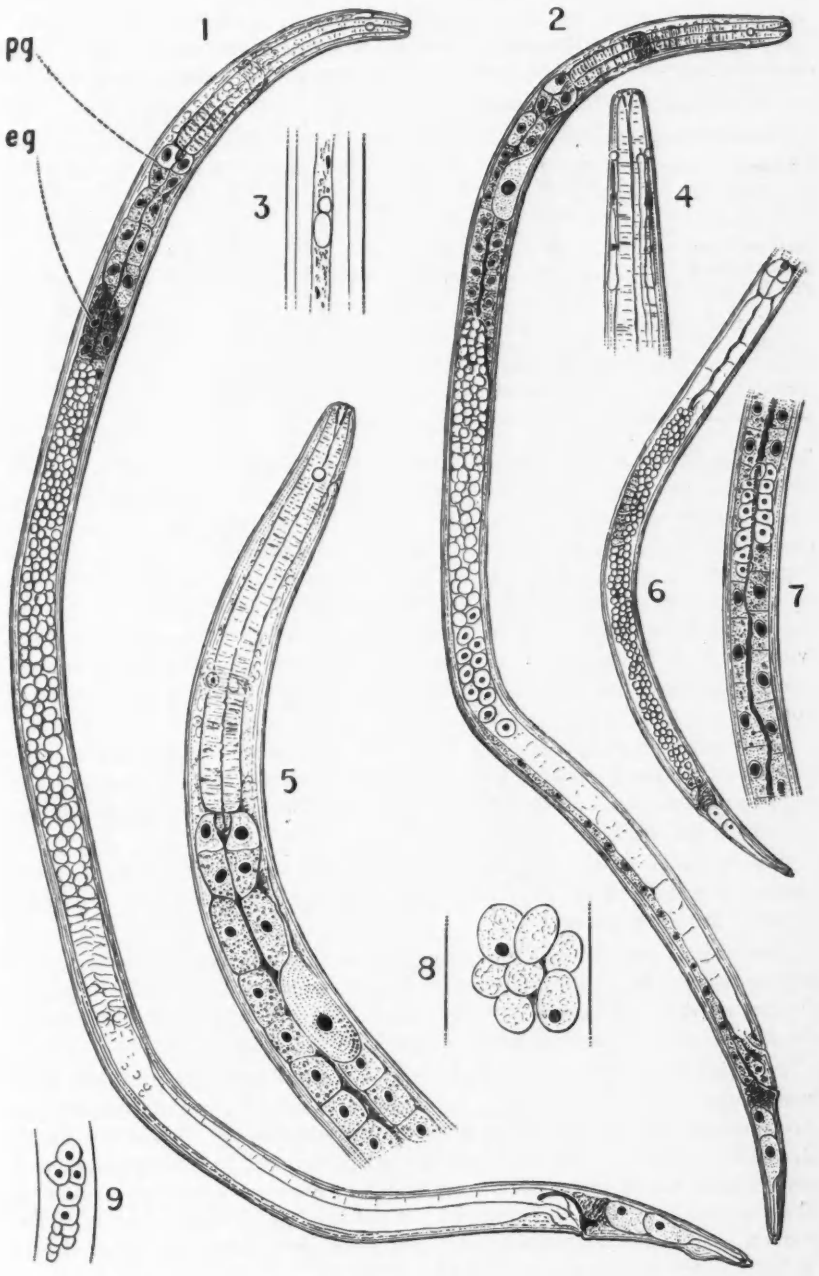
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TABLE I

MEASUREMENTS OF TWO FEMALES, ONE MALE, AND 11 LARVAE OF *M. cameroni*

	Total length, mm.	a	β	γ	v
1st female	0.792	30	7	13	88%
2nd female	0.760	38	6.5	9	86%
Male	1.054	40	7.3	11	Spicula 38 μ
Smallest larva	0.380	24	4	6.4	
Preadult larva	0.676	31	6	8.6	
Preadult larva	0.702	32	7	9.5	
Myers furnished the following measurements:					
Female	0.98–1.10				
Male	0.83–0.95				
Larvae	0.13–0.800				

lateral longitudinal chords visible through cuticle, about two-thirds as wide as body, consisting of three longitudinal series of cells. Amphidial opening twice to more than three times width of head behind head end, circular, quite deep (Figs. 4, 10, 11), diameter 3 μ in female; in male, slightly larger (compare Fig. 10 with Fig. 11); amphidial duct as shown in same figures with oblong sensilla and rather few terminals. Excretory gland prominent, from two to more than three body widths behind anterior end of intestine (compare Fig. 1 with Fig. 2), with large nucleus; excretory canal leading to an ampulla located ventrad of amphidial circle; a very fine outlet duct connecting ampulla to body surface.

Head with six rather obscure lips, each with small but distinct papilla; posterior of these a circle of 10 short, minutely setose, cephalic papillae, the latero-submedial ones minutely longer than their dorso- and ventro-submedial partner (Figs. 10, 11).

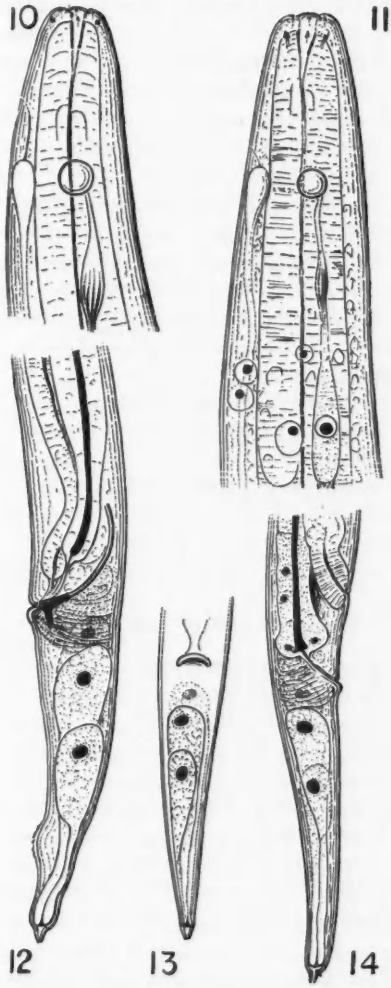
Oral entrance leading to short but quite wide vestibulum; buccal cavity conical, about 8 μ long, anterior half with distinct sclerotized wall; denticles or other armature absent.

Oesophagus completely surrounding buccal cavity, nearly cylindrical, with strong radial and obliquely radial musculature; nerve ring rather obscure, located at about two-thirds of length of oesophagus; latter joined to intestine by distinct subconical to oblong–spheroidal cardia (Fig. 5).

Intestine beginning with a progaster, i.e. a setoff group of four cells (proventriculus of Paramonov (16)), two anterior ones clear, not granulated, two posterior ones dark brown, finely granulated like rest of intestinal cells (Figs. 1, 2, and 5); number of intestinal cells about 50, arranged in two series; all cells uninucleate, nuclei large; anterior cells shorter than posterior ones; a distinct rhabdionate layer ("Stäbchensaum", "bacillary layer") lining part of rather narrow intestinal canal; contents of latter not identifiable, but showing particles of different size, some as shown in Fig. 3.

Rectum well set off, short, with an aggregate of cells (rectal glands) and muscle fibers (dilatatores ani). In some specimens posterior anal lip protruded; anus as shown in Fig. 13.

Tail (Figs. 12–14) with two large, very distinct, clear foot gland cells; a third, the most anterior one, obscure, reduced in size, easily overlooked,



FIGS. 10 to 14. *Monhystera cameroni* n. sp.
FIG. 10. Head end of male; compare size of amphid and outlet of excretory gland with that of female in Fig. 11. 1333×
FIG. 11. Head end of female. 1333×
FIG. 12. Tail end of male with copulatory apparatus and foot glands. 315×
FIG. 13. Ventral view of tail end of female to demonstrate wide anal opening. 315×
FIG. 14. Tail end of female in side view to demonstrate location of vulva and vagina and arrangement of foot glands. 315×

covered by complex of anal and rectal cells and muscles. Outlets of the three cells forming separate distinct ampullae at short, conical, terminal outlet tube (spinneret or adhesive tube). Canal and ampulla of most anterior cell narrower and smaller than those of two others. Tail tip not swollen.

A somewhat similar situation is reported by Allgén (2) for *Monhystera tasmaniënsis* Allgén, 1927. This author, however, states that in his species there were actually only two foot gland cells present, a statement which Paramonov (16) considers to be erroneous.

Female sexual organs prodelphic, posterior branch completely absent. Vulva rather obscure, a transverse slit, about one vulvar body width or less in front of anus, i.e. at 86–88% of body length. Vagina much less prominent than rectum, short, directed forward, forming a tube with a thick muscular wall.

Only two young females were available for study; their gonads were obscure. But Myers, in a sketch of a stained female specimen sent to me shows a reflexed, narrow, and short germinal end portion at the anterior end of the gonad (this is not shown in Fig. 2). She further mentions a tubular oviduct and uterus with a section functioning as receptaculum seminis. She describes the eggs as large, oblong, of about 50 μ in length and as deposited unsegmented, often adhering to debris.

Ovary and outlet to the right of the intestine.

Male with broad testis, containing massed spermatocytes, also entirely located on right side of intestine.

Spicula thin, almost setose, curved, distal portion laterally enveloped by lamellae of gubernaculum; latter with short posteriad-directed apophysis (Fig. 12). Myers' sketch of the testis also shows a short and narrow reflexed end portion which I was not able to see in the single male specimen submitted.

Relationship of New Species

Monhystera cameroni is not closely related to any of the previously mentioned commensalistic monhysterids but quite closely resembles *Monhystera socialis* Bütschli, 1874, a form collected by Bütschli in a basin with brackish water at Kiel, Germany, where it occurred in large numbers in tufts of oscillatorias (Bütschli calls them "Oscillarienmassen") growing in abundance in these putrid waters during the summer. Bütschli tells of putting clumps of these algae into glass jars filled with water, and of then having seen enormous numbers of these nematodes emerging from the algal masses forming weblike accumulations on the glass walls or in the water between the masses of algae, or aggregating otherwise as clumps and knots of intertwined specimens.

Ditlevsen (11) states that *M. socialis* is a rather common littoral form in Danish coastal waters on putrefying seaweed, and Allgén (4) reports the species from Hallands Väderö (Zostera region, at depths of 0–5 m.) and from various locations in the Öresund (between Sweden and Denmark). However, the description given by the same author in 1935 (5) is quite at variance with that imparted by Bütschli (7), particularly in regard to the spiculum and gubernaculum. Schuurmans Stekhoven (18, 19) mentions the occurrence of *M.*

socialis in the Zuidersee. All these records are from the North Sea and Baltic Sea. In 1951, Chitwood (9) reported *M. socialis* from a new region, namely *Sargassum* of a fish trap at Cedar Bayou, Texas, thus seemingly demonstrating that this European species occurred also on the Texan shore. However, a comparison of Chitwood's description with that given by Bütschli shows the two forms to be different at least in the following characters which may serve as diagnostic:

1. *M. socialis* of Bütschli is a much larger form: females measure 1.9–2.2 mm. compared with 1.2 mm. of the female and 1.4 mm. of the male in Chitwood's specimens; likewise the tail is shorter in the former, $\gamma = 10$–11 in the female, 14 in the male as compared with 8.2 in the female and 9.8 in the male of the latter.

2. In *M. socialis* of Bütschli the vulva is much closer to the anus, only about one vulvar body-width in front of the anus or at 89% of the total body length; in Chitwood's specimens it is at 76% or about four times the body width in front of the anus.

3. *M. socialis* of Bütschli lacks the four submedial postcephalic setose papillae that Chitwood figures for the Texan form.

4. *M. socialis* of Bütschli has a short caudad-directed apophysis on the gubernaculum which is absent in Chitwood's form.

5. Bütschli mentions and draws a number of cells at the posterior end of the oesophagus, cells that appear to be absent in the Texan species.

It is certain that a more detailed study of both forms will show additional differences.

Wieser (20), in his review of the monhysterids, obviously relied on Chitwood's erroneous identification instead of Bütschli's original description of the type since he placed *M. socialis* in his group of monhysteras that have a gubernaculum without a posterior apophysis.

In order to prevent further confusion it is proposed to establish Chitwood's *Monhystera socialis* as a separate species: *M. chitwoodi* n. sp., with the differentiating characters just mentioned above.

It is necessary to call attention to this situation here because of the close relationship of *M. cameroni* to both of these species, with which it belongs in a group of monhysterids characterized by the presence of a progaster, i.e. of two clear cells followed by two pigmented and granulated cells at the anterior end of the intestine, the four being set off from the following portion of the intestine by a broad constriction. This progaster is interpreted as the initial stage in the formation of a "pseudobulb" as seen in the genera *Monhystrium* and *Tripylium* mentioned above. The functional significance of this structure is unknown but as a morphological feature it might be of value at the time when an improved classification of the monhysterids is established, a classification based on morphological resemblance and lines of evolution. There are other characters that unify these forms, such as the structure of the gonads, the slender, arcuate spicula, the form of the buccal cavity, the pattern formed by the labial and cephalic papillae, and the shape of these organs, etc.

Diagnosis of Monhystera ***cameroni*** *n. sp.*

Monhystera resembling *M. socialis* Bütschli, 1874 nec. Chitwood, 1951 but differs from the former by: (*a*) smaller size, 0.792–1.024 mm. as compared with 1.9–2.2 mm.; (*b*) cells at the posterior end of the oesophagus mentioned and figured by Bütschli for *M. socialis* absent; (*c*) circular amphidial openings two to three headwidths behind head end as compared with less than a head width in *M. socialis*; (*d*) only two prominent foot gland cells of normal size, not three as in *M. socialis*, the third being obscure and dwarfed; (*e*) male with ventral copulatory hump in front of tail end, not known for *M. socialis*.

M. cameroni differs from *M. chitwoodi* (= *M. socialis* Bütschli of Chitwood, 1951) by: (*a*) position of vulva closer to anus (86–88%), in *M. chitwoodi* at 76%; (*b*) position of amphidial opening, in *M. chitwoodi* only about one head width from anterior end; (*c*) a copulatory hump on the male tail, instead of a preanal papilla as in *M. chitwoodi*; (*d*) gubernaculum in *M. chitwoodi* without posteriad-directed apophysis; (*e*) absence of circle of four postcephalic submedial setose papillae which are present in *M. chitwoodi*; (*f*) presence of 10 cephalic papillae instead of six cephalic setae as seen in *M. chitwoodi*.

Discussion

Osche (15) has reviewed and discussed to some extent the association of nematodes and crustaceans as presently known. He points out that this association in some instances may be purely accidental but that in others it has developed to a status of permanence where the nematode is found only as commensal, no longer as "free-living". We are unable to state if *M. cameroni* belongs to the latter or the first group.

Monhysteras are particularly fitted for a life on maxillipeds, mandibles, pleopods, etc., as well as in the gill chambers of crustaceans, because of their foot gland system which is very effective. Furthermore, they are excellent swimmers. These two features have enabled some forms to live in tufts of algae, mosses, and other plants in streams with strong currents and in waterfalls as well as in tidal zones. At least some of the forms need water rich in oxygen. These are conditions that a habitat on crustaceans also offers.

As mentioned previously, of the three foot glands of *M. cameroni* only the two posterior ones are immediately noticeable; they are large, of a clear and spongiose structure, while the third and most anterior one is small, of opaque appearance, and easily overlooked. Its function as a supplement to the two others may be doubtful. Obviously its reduction is compensated for by the two oversized posterior ones. But the fact that one of these glands may be reduced or may have become vestigial is of theoretical interest. It demonstrates that reductions in the foot glands do occur even in forms in which adhesive ability is all important and is fully retained in spite of a partial reduction, as in the present case. Here the reduction of one of the glands is obviously compensated for by the increased size and consequently the correlated increased functional efficacity of the remaining two.

For reasons of clarity and comparative morphology, it is proposed to replace the term "caudal" glands heretofore used to designate the adhesive or haptic caudal glands characteristic of many of the aphasmidian nematodes with the term "foot" glands, a designation widely used for the functionally similar glands of other Aschelminthes (see also Hyman (13)). The use of the term caudal glands is objectionable because there are other types of glands in nematodes that are caudal, e.g. some of the hypodermal glands as in *Anaplectus*; there are the rectal glands and the phasmidial glands. Furthermore the "caudal" glands of authors actually may be precaudal as in numerous marine nematodes (*Oncholaims*, *Thoracostomas*). Paramonov (16) in this instance uses the terms "incaudal" and "excaudal" caudal glands, both very undesirable designations. Neither are terms such as "haptic" glands and "adhesive" glands satisfactory because nematodes possess other kinds of haptic or adhesive glands, to mention only the Draconematidae, where glands of this type of function occur in the ventro-preanal and in the dorsocephalic region.

It is highly desirable that more attention be given to the various gland systems occurring in the tail of nematodes. Of particular need is a comparative study of the distribution, morphology, and function of the foot glands and the phasmids, and their obliteration and disappearance in the various nematode groups. The fact that phasmidial and foot glands never occur together, that the presence of the one excludes that of the other, and furthermore, that the appearance of phasmids with a change to terrestrial and parasitic life, when foot glands become unnecessary, suggest that these gland types are homologous. Phasmidial glands are modified, functionally and numerically, reduced foot glands with laterally shifted outlets. With this perspective it will be possible to demonstrate and correlate a number of interesting lines of resemblance and evolution in nematodes.

Special mention should be made of the amphids of *M. cameroni*; as far as I know it is the first instance in which part of the internal structure of this organ has been ascertained in a *Monhystera*. A resemblance of these structures in *Monhystera* to those seen in the plectids is evident.

A feature to be given more consideration in a future classification of the monhysterids is the form and structure of the gonads. There exists a remarkable difference in this regard, e.g. between the fresh-water forms of *Monhystera* and some of their marine relatives (as shown here for *M. cameroni*). In the fresh-water monhysterids there is a strictly monoserial arrangement of the oocytes; in *M. cameroni* and some other marine members a broad polyserial or massed order. In the former there is a late development of the gonads before the last molt, in the latter (as shown in Figs. 7 and 9) numerous primitive gonad cells occur in all larval stages, their number increasing with progressing development.

A Microsporidial Parasite of Monhystera cameroni

Three of the larval specimens in the collection of *M. cameroni* here studied were obviously infected by a sporozoan parasite. Figures 6 and 8 show details

of this infection. In the main it appears to be a parasite of the body cavity (Fig. 8). One stage of the parasite however appeared to be in the intestinal cells. The body cavity stage shown in Fig. 6 measures about 4–6 μ and is of slightly oblong form.

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THE NORMAL BLOOD CHEMISTRY OF THE BEAVER (CASTOR CANADENSIS)

A. PACKED-CELL VOLUME, SEDIMENTATION RATE, HEMOGLOBIN, ERYTHROCYTE DIAMETER, AND BLOOD CELL COUNTS[1]

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Abstract

Packed-cell volume, sedimentation rate, hemoglobin, red blood cell count, white blood cell count, differential count, and red cell diameter have been determined on blood samples taken from 22 beavers. The various values obtained are in general comparable with those reported for the other rodents. Exceptions to this general agreement were noted in the much higher sedimentation rate and slightly larger red cell diameter of beaver blood.

Introduction

There is strong field evidence to suggest that the nutritional status is one of the major factors controlling beaver population density and hence colony survival. It becomes important therefore to understand the nutritive needs for normal growth and reproduction and to establish some of the parameters that can be used to indicate departure from this normal state. Accordingly this laboratory has been studying some aspects of beaver nutrition. The present report records certain "normal values" for some of the blood constituents. Similar values for other blood components will be reported later.

Methods and Materials

A. Experimental Animals

Fifteen adult and seven yearling beavers (*Castor canadensis leucodontus*) were obtained during the period from October 1954 to October 1955 from the Courtenay area on Vancouver Island. The animals were caught in Bailey live traps and transported by air to the laboratory as soon as possible after capture.

B. Housing

Special pens were constructed to house the experimental beaver. Since little is known about the management requirements for captive beaver, an attempt was made to simulate the natural environment within the rather rigid limits imposed by experimental regimens. The description of the special pens and the management procedures followed will be reported later.

C. Blood Samples

Blood samples were obtained by heart puncture, from animals immobilized in a holding cone. Two samples were collected from each animal during an interval of 2 months.

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D. Analytical Procedures

(*i*) *Packed-cell Volume*

The Wintrobe method (4) was used for the determination of packed-cell volume. The oxalated blood was centrifuged in a hematocrit tube at 3000 r.p.m. for 30 minutes.

(*ii*) *Sedimentation Rate*

The modified Westergen method (4) was employed. Analyses were carried out within one hour of drawing the blood samples. The level of red blood cells was determined after the samples had been allowed to stand in the tubes for 1 hour.

(*iii*) *Hemoglobin*

The method of Wong (8) was used without modification.

(*iv*) *Blood Cell Counts*

Red, white, and differential blood cell counts were conducted using the methods outlined by Perry and Morgan-Dean (4).

(*v*) *Erythrocyte Diameter*

Red cell diameters were obtained from 543 measurements made on the blood from 18 beavers. The cells were stained with Wright's stain and examined under oil immersion using a standardized ocular micrometer to measure cell diameter.

(*vi*) *Statistical Analysis*

Statistical analyses of the data obtained were performed by the method of Snedecor (5).

Results and Discussion

(*a*) *Packed-cell Volume*

The hematocrit value or packed-cell volume affords a relative measure of the erythrocyte level of whole blood. For this reason it is usually considered to be one of the most useful values to permit an interpretation of the nutritional status of the hematopoietic tissues. The values presented in Table I suggest that the erythrocyte level in normal beaver blood is comparable to that (42%) reported for the rabbit and pig (2). The level is lower than that found in man and the dog (45%) but higher than for the horse, sheep, and goat (34%) (1,6). The packed-cell volume for the yearling beaver is slightly below that of the adults but the difference is not significant. Kitts *et al.* (3) have reported higher values ($58.2 \pm 1.33\%$) for the adult black-tailed deer. Within the rodents, values of 46% for the hamster, 39.8% for the rabbit, and 39.4% for the rat have been reported (1,6). The beaver values presented here are therefore similar to those given for other species of rodents.

(*b*) *Sedimentation Rate*

There is considerable variability (S.D. 22.5) in the values obtained for the sedimentation rate of beaver blood. This variability occurs in samples taken

TABLE I

SOME NORMAL BLOOD VALUES* FOR THE BEAVER (*Castor canadensis leucodontus*)

	Yearling beaver			Adult beaver			Yearling and adult beaver		
	Mean and standard error	Standard deviation	Range	Mean and standard error	Standard deviation	Range	Mean and standard error	Standard deviation	Range
Packed-cell volume (%)	40.3 ±0.6	2.6	36–45	42.1 ±0.5	2.0	40–46	41.2±0.4	2.5	36–46
Sedimentation rate mm./hr.)	34.7 ±6.5	22.5	9–72	40.1 ±7.3	23.2	10–78	36.6 ±4.8	22.4	9–78
Hemoglobin level (g./100 ml.)									
A. Summer level	12.8 ±0.2	0.6	12.3–14.2	13.5 ±0.2	0.7	12.6–14.7	13.1 ±0.2	0.7	12.3–14.7
B. Autumn level	13.6 ±0.4	1.4	12.0–16.0	14.0 ±0.4	1.1	11.1–15.8	13.8 ±0.4	1.3	11.1–16.0
Cellular elements									
A. Red cells (millions/mm.^{3})	4.22±0.21	0.72	3.7–5.0	4.35±0.10	0.35	3.8– 4.9	4.28±0.11	0.55	3.7– 5.0
B. White cells (thousands/mm.^{3})	14.6 ±1.01	3.5	7.6–18.2	11.6 ±0.9	3.1	7.0–16.7	13.2 ±0.7	3.6	7.0–18.2

*Based on determinations on a total of 22 beavers sampled twice over a 6-month period.

from different animals as well as in samples from the same animal taken at different times. This is understandable in view of the established fact (7) that sedimentation rate varies with packed-cell volume and with sex and age, as well as with the presence or absence of certain pathological conditions. In the present study it was not possible to carry out the extensive work necessary to establish the relationship between pathological state and sedimentation rate. For present purposes it seems safe to conclude that the mean value of 34.7 ± 6.5 mm./hour obtained here can be taken as representative for normal beaver. It is of interest to note that this value is considerably higher than those reported for the other rodents. It is of the order reported for the horse, and thus among the highest normal rates known in the mammals.

(*c*) *Hemoglobin*

Data for the different age classes are summarized in Table I. It will be noted that the summer values were significantly lower than those obtained from the fall determinations. The levels for the summer yearlings are significantly lower than those for the adults.

A definite change in hemoglobin level associated with growth and development has been well documented for man and for certain domestic animals (2). In these, following an initial high level shortly after birth, the hemoglobin level declines and then during the early phases of the animal's life it gradually increases until shortly after puberty. Kitts *et al.* (3) have reported a similar pattern of changes in the hemoglobin level of growing deer. Postpubertal males have slightly higher levels than females of corresponding age. The beaver appears to have the same hemoglobin level as the other mammals.

(*d*) *Erythrocyte Counts*

The red cell counts given in Table I are lower than those reported for most of the other mammals including the rat, mouse, and hamster. For example, most of the other mammals have counts ranging from 6 to 10 million/mm.^{3} (1, 6). The beaver and the guinea pig seem to be somewhat unique in that the reported values are all below 5 million/mm.^{3}. Despite this lower count it has been shown above that the hemoglobin level for this species is the same as that for those species with higher cell counts. As might be expected the same applies to the hematocrit values. A partial explanation of this may be found in the size of the cells in beaver blood.

TABLE II

THE DIAMETER OF THE BEAVER ERYTHROCYTE (DRY FILM PREPARATION)

	Yearlings, μ	Adults, μ	All beavers, μ
Mean and standard error	7.6 ± 0.1	7.6 ± 0.1	7.6 ± 0.1
Standard deviation	1.2	1.2	1.2
Range	6.0–9.7	6.0–9.7	6.0–9.7

The diameter of the beaver erythrocyte as measured on dry films is given in Table II. In both the adult and young the cells average 7.6 μ. The comparable figures for other species are: dog 7.0; guinea pig 7.4; hamster 5.6; mouse 6.0; rat 7.5 μ. (1, 6). It is possible that this slightly larger than average erythrocyte is in some way related to the needs of an aquatic mammal.

(*e*) *White Cell and Differential Counts*

The results of the white cell counts are given in Table I. The normal (7) range of leucocyte count in man is between 5 and 10 thousand per cu. mm. Dukes (2) reported that leucocyte counts for the horse, cattle, sheep, and goat range from 5 to 14 thousand per cu. mm. while the pig, dog, and cat have normal ranges above 20 thousand per cu. mm. The range for the beaver was found to be between 7 and 18 thousand per cu. mm.

Values for yearling beaver are significantly higher than those for adults. The results of the differential cell counts are summarized in Table III.

TABLE III

SUMMARY OF DIFFERENTIAL CELL COUNTS FOR ALL BEAVER EXPRESSED AS A PERCENTAGE OF THE LEUCOCYTE COUNT

	Neutrophiles	Eosinophiles	Basophiles	Lymphocytes	Monocytes
Mean	63.4	2.4	0.6	29.8	3.5
Medium	66.0	2.0	1.0	30.0	3.0
Range	52–71	1–5	0–2	23–41	1-7

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CRYPTIC SPECIATION IN THE VERNALIS GROUP OF CYCLOPIDAE[1]

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Abstract

Breeding experiments performed on the descendants of 30 separate populations of *Cyclops vernalis* Fischer show a pattern of positive and negative results indicating the existence of seven reproductive isolates. Most of these isolates vary morphologically, but they differ in the nature and degree of this variation. They are, for the most part, not easily indentifiable but the one which varies the least is shown to be referable to *C. brevispinosus* Herrick, a species which had been placed in the synonymy of *C. vernalis*. No attempt is made to name the six other isolates.

In addition to their experimentally demonstrated reproductive isolation and differences in morphological variability, the isolates are regarded as species on the basis of differences in their ability to serve as first intermediate hosts for the tapeworm *Triaenophorus crassus* Forel.

Introduction

The family Cyclopidae (Crustacea, Copepoda) has achieved notoriety, among biologists who must deal with this group in their studies, for being "difficult" taxonomically. Part of this difficulty arises in distinguishing species from varieties or other infraspecific categories. Differences of opinion regarding the assignment of morphological variants to the species or subspecies level add to the confusion. This confusion is well illustrated by the species *C. vernalis* Fischer, the subject of the present paper. Taxonomists have relied mainly on morphological discontinuity as a species criterion, using subjective reasoning for delimitation of species. This has led to the sort of tangled nomenclature outlined for *C. vernalis* in the final section.

It is a frequent observation that matings between members of different species are inhibited, or as appears to be the case in Cyclopidae, prevented by little known factors. Dobzhansky (1941 (3)) defines a species as the "stage of evolutionary process at which the once actually or potentially interbreeding array of forms becomes segregated into two or more separate arrays which are physiologically incapable of interbreeding". It will be shown here that the demonstration of reproductive isolation can be used as a valuable tool where the search for diagnostic morphological characters, usually associated with speciation, fails or at least presents difficulties. The technique consists of placing Cyclopidae in an experimental situation where they sort themselves into species on the basis of the capability of breeding, or the lack of it.

The reproductive barrier between species of Cyclopidae has so far appeared to be invincible. Efforts to cross different species have been made by Neubaur (1913 (10)), Lowndes (1929 (6) and 1932 (7)), Coker (1934 (2)), and in the present study, always without success. Hybridization seems not to occur in the Cyclopidae.

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In the present study, controlled breeding experiments have been performed on descendants of 30 populations of *Cyclops vernalis*, mostly from Eastern Canada. The results have indicated the existence of seven reproductively isolated species or "isolates" in these populations. The isolates have been compared with one another as to distribution, ecology, morphology, and physiology, in a search for differences between them which might corroborate their species status and serve to identify them.

Evidence of Reproductive Isolation

Experimental Procedure

As a test for reproductive isolation between allopatric populations the following method was used. One or more cultures were begun with an individual ovigerous female from each natural population. Each culture was therefore known to be monospecific and as genetically homogeneous as practicable. Ovigerous females were selected from these cultures and used to begin the breeding experiments.

For a typical breeding experiment a female from each culture to be tested was isolated in a 10 ml. watch glass. When her eggs hatched, the female was removed and the resulting nauplii allowed to grow up to the first copepodite stage. Individual first copepodites were then isolated in watch glasses and reared to maturity. The copepods were fed *Paramoecium* throughout the experiment.

For the test of crossability males and females were placed together in pairs, a male from one population and a female from another, one pair to a 10 ml. watch glass. In the controls both members were from the same population or were otherwise known to belong to the same isolate. The pairs were examined daily for a week and production of eggs and nauplii noted.

Results

The results indicate that seven reproductive isolates exist in the 30 populations used in breeding experiments. These isolates were designated by the letters A to G, in the order in which they were discovered. The total number of crosses between and within isolates and their results are given in Table I. A total of 305 attempts were made to cross different isolates, all with negative results. Within isolates 241 crosses were tried, of which 176 or 73% had positive results, that is, the female laid fertile eggs within one week. Of the crosses within isolates, 106 were between separate populations, and of these 79 were positive.

It was found that 70 to 80% of the conspecific pairs that reproduced did so before the end of the third day. Most of the fertile pairs produced their first eggs on the second day after being placed together. Figure 1 shows a plot of the number of pairs of isolates A and B reproducing on each day after the pairs were set up. The peak was reached on the second day in both isolates and by the third day most of the results were in. It is therefore apparent that 1 week was a sufficient waiting period.

TABLE I

TOTAL NUMBER OF BREEDING EXPERIMENTS AND RESULTS OF CONTROL CROSSES. NUMBERS OF PAIRS REPRODUCING ARE GIVEN IN PARENTHESES. ALL EXPERIMENTAL CROSSES WERE NEGATIVE

Isolates	A	B	C	D	E	F	G
A	101 (80)	51	33	16	14	24	15
B		71 (60)	28	25	13	12	7
C			13 (8)	7	1	3	1
D				31 (17)	9	8	6
E					8 (5)	19	4
F						14 (5)	9
G							3 (1)

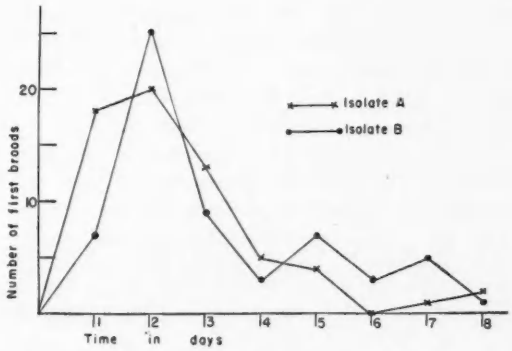

FIG. 1. Time of first spawnings of pairs of isolates A and B. Most pairs produced their first batch of fertile eggs on the first, second, or third days.

In order to identify each new collection of *C. vernalis* it was necessary to try crossing it with each of the already established isolates. It was invariably found that if it would cross with any of the isolates it would cross with only one. Tables II and III illustrate the method of assigning a new population to an isolate. In Table II the descendants of a population in a pond on Cape Breton were found to breed only with known isolate A. Attempts to cross this form with the other isolates were all unsuccessful. Table III illustrates the situation where descendants from a new collection would not cross with any of the known isolates, necessitating the setting up of a new isolate.

The observation that the results of breeding experiments between 30 populations emerge as a pattern suggesting the existence of seven isolates instead of a random assortment of positives and negatives is significant. Let us consider four populations, *a*, *b*, *c*, and *d*. If *a* breeds with *b* and also with *c*,

TABLE II

IDENTIFICATION OF A NEW COLLECTION, "CAPE BRETON", WHICH BELONGED TO AN ISOLATE ALREADY ESTABLISHED (A)

Cross	No. of pairs	No. that reproduced
Cape Breton × Cape Breton	6	5
Cape Breton × A	9	5
× B	4	0
× C	1	0
× D	2	0
× E	3	0
× F	5	0
× G	5	0

TABLE III

IDENTIFICATION OF A NEW COLLECTION, "SPROULE PORTAGE", WHICH DID NOT BELONG TO AN ISOLATE ALREADY ESTABLISHED, NECESSITATING THE INSTATEMENT OF A NEW ISOLATE (E)

Cross	No. of pairs	No. that reproduced
Sproule Portage × Sproule Portage	8	5
Sproule Portage × A	14	0
× B	13	0
× C	1	0
× D	9	0
× F	19	0
× G	4	0

b and *c* are found to interbreed. On the other hand, if *d* will not breed with *a*, neither will it cross with *b* or *c*. Therefore *a*, *b*, and *c* can be considered as belonging to one isolate, and *d* to another. No anomalous results were encountered.

Nature of the Reproductive Barrier

This is a subject which was not especially investigated in the present study. Two observations were made, however, which suggest the direction future studies of reproductive isolation in Cyclopidae should take. First, females of non-conspecific pairs usually did not extrude eggs. Only 19 out of the 305 females with non-conspecific males did so, and in these instances the eggs invariably were few, and they disintegrated without hatching. Secondly, empty spermatophore cases were occasionally seen on females which had only been in the presence of non-conspecific males.

The significance of these observations can be understood in the light of what normally occurs in the reproductive cycle of Cyclopidae. The extruded fertilized egg takes only 1 day to develop at temperatures above 20° C., and hatches into a free-swimming nauplius. Ontogeny of an individual consists of 12 stages separated by 11 molts. The first six stages are termed nauplii, the remainder copepodites. This entire development from egg to adult may

require only 10 days, an average of more than one stage per day. The adults are dioecious with considerable sexual dimorphism. Males are smaller than females, and have modified antennae. Located in the genital segment of the male, a conspicuous bilobed seminal vesicle secretes the spermatophore, a sperm mass in a chitinous case. This spermatophore is placed by the male on the genital segment of the female, where it adheres. The sperm mass is released by rupture of the case, and moves into the seminal receptacle of the female. The empty case may remain for a time, indicating that the female has been inseminated, but it eventually drops off.

Each male is capable of producing several spermatophores, and therefore of fecundating more than one female, but males are short-lived after reaching maturity. The inseminated female may extrude her first clutch of eggs within one day, and succeeding clutches every other day thereafter. The eggs are produced in ramifications of the oviducts in the cephalothorax dorsal and lateral to the intestine. These oviducts communicate with the outside by openings in the genital segment at the seminal receptacles. The eggs are thought to be fertilized (Wesenberg-Lund, 1939 (12)) as they pass the sperm mass in the seminal receptacle. Until they hatch, the eggs are carried by the female in two sacs attached to the genital segment.

The observations that males at least sometimes affix spermatophores to females of other species, and that females of non-conspecific pairs, like females kept in isolation, seldom lay their unfertilized eggs, suggest three possibilities. First, perhaps the sperm mass does not enter the seminal receptacle of a female of the wrong species, even though the spermatophore has adhered. Secondly, the sperm mass may enter, but must be conspecific to stimulate extrusion of the eggs. The third possibility is that fertilization of the eggs occurs in the cephalothoracic branches of the oviducts and only fertilized eggs are extruded. It can, at any rate, be concluded that behavioristic incompatibility of the non-conspecific pair is not always, and perhaps never is, the isolating mechanism involved.

Distribution and Ecology of the Isolates

Most of the bodies of water from which material for the breeding experiments was collected were in the Toronto region, where the experimental work was done, but some collections were from elsewhere, either brought or sent by other investigators or obtained by excursion. A list of the localities sampled and the isolate yielded by each collection is given in Table IV.

In an attempt to characterize each isolate ecologically the bodies of water are classified as lakes, rivers, or ponds, and the ponds as permanent or temporary. Cyclopidae can also be described as being planktonic, i.e. free-swimming and inhabiting open waters, or non-planktonic, i.e. usually moving about on surfaces. Isolate C is clearly separable from the six others on being the only planktonic isolate and being found only in lakes and permanent ponds. Isolates A and B, which were the only others found frequently enough to warrant conclusions on their ecology, occurred in both permanent and temporary waters.

TABLE IV

DISTRIBUTION OF THE SEVEN ISOLATES IN 30 HABITATS

Habitat No.	Isolate	Name of body of water or nearest community	Type of habitat
1	A	Maple, Ontario	Permanent pond
2	A	Credit Forks, Ont.	Temporary pond
3	A	King, Ont.	Permanent pond
4	A	King, Ont.	Permanent pond
5	A	Toronto, Ont.	Permanent pond
6	A	Caledon, Ont.	Temporary pond
7	A and B	Barrie, Ont.	Permanent pond
8	A	Algonquin Park, Ont.	Permanent pond
9	A	Cape Breton, Nova Scotia	Permenent pond
10	B	Sault Ste. Marie, Ont.	Permanent pond
11	B	Clear Lake, Ont.	Lake
12	B	Brampton, Ont.	Permanent pond
13	B	South River, Ont.	River
14	B	Credit River, Ont.	River
15	B and C	Opeongo Lake, Ont.	Lake
16	B	Sault Ste. Marie, Ont.	Temporary pond
17	C	Kearney Lake, Ont.	Lake
18	C	Clarke Lake, Ont.	Lake
19	C	Haynes Lake, Ont.	Lake
20	C	Lake-on-the-Mountain, Ont.	Lake
21	C	Heming Lake, Manitoba	Lake
22	C	Tracy City, Tennessee	Permanent pond
23	C	Brampton, Ont.	Permanent pond
24	D	Maple, Ont.	Permanent pond
25	D	Wilcox Lake, Ont.	Permanent pond
26	D	King, Ont.	Temporary pond
27	E	Algonquin Park, Ont.	Permanent pond
28	F	Lac Fortune, Quebec	Lake
29	F	King, Ont.	Permanent pond
30	G	Toronto, Ont.	Temporary pond

There is not enough evidence from these collections to delimit the geographic distribution of each isolate, but some observations are significant. Isolates A and C are apparently widespread on the North American continent, A occurring not only in Ontario but also on the east coast (Cape Breton); and C being found in samples from Heming Lake, Manitoba, and an impoundment in Tennessee, as well as several localities in Ontario.

The problem of whether more than one isolate can occur in a single body of water has not been especially studied, although some evidence has emerged. Habitat No. 7 (Table IV), a pond near Barrie, Ontario, yielded both isolates A and B, and in habitat No. 15, a lake in Algonquin Park, Ontario, both isolates B and C were found. On the other hand, habitats 1, 5, 19, 24, 27, and 30 were sampled more than once, and always the same isolate was rediscovered in each.

Morphology of the Isolates

In addition to reproductive isolation, and perhaps because of it, a degree of morphological discontinuity generally characterizes species, even those belonging to a multifarious group. We have come to expect that every species must have a description which sets it apart from all others, both to lend support to the thesis that it really is a species and to make its identification and use possible. Accordingly, a search has been made for characters which might distinguish the *Cyclops vernalis* isolates from one another.

The degree of morphological variation found within certain of the isolates made it necessary to examine as many individuals as possible. This variation is at least partly temperature-caused. In brief, isolates reared at high temperatures tended to vary, even among siblings, while at low temperatures intraspecific variation was slight.

Cyclopidae fairly bristle with morphological characters which have possible taxonomic significance. The characters studied in the present work have been limited mainly to those already in use in cyclopid taxonomy. These include the number of spines and setae on the swimming legs, the relative lengths of the two spines on the terminal segment of the endopodite of the fourth leg, the shape of the basal segment of this same ramus, the relative lengths of the furcal setae, and total body length. No useful differences were found in the antennae, fifth leg, or in the length or shape of the furcal rami.

Swimming Leg Armature

Variation here consists of the presence, absence, or interchangeability of spines and setae. The difference between these two structures should first be explained. Figure 2 illustrates a typical spine and seta. The spine is short, inflexible, and beset with fine denticles and notches along two edges. A seta is long, flexible and instead of teeth has two rows of long hairs lying in

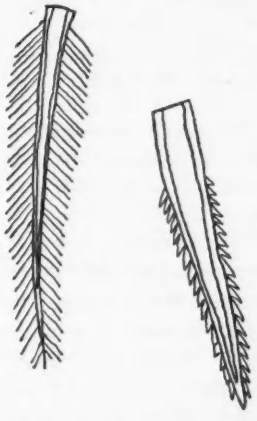

FIG. 2. Typical seta and spine of swimming legs of *Cyclops vernalis*. The seta is slender and flexible and has fringes of fine hairs. The spine is more robust and has fringes of denticles. (Traced from photomicrograph.)

the same plane. Two sorts of intermediates between spines and setae occur, but these are rare and are discussed under "anomalies". Otherwise the structure at a given place is easily identified as a spine or a seta.

In this group of Cyclopidae, the four pairs of thoracic legs are alike in segmentation. Each leg consists of two rami, termed endopodite and exopodite, of three segments each. Variation was rarely found in the armature of the proximal and middle segments of either ramus of any leg. In all specimens

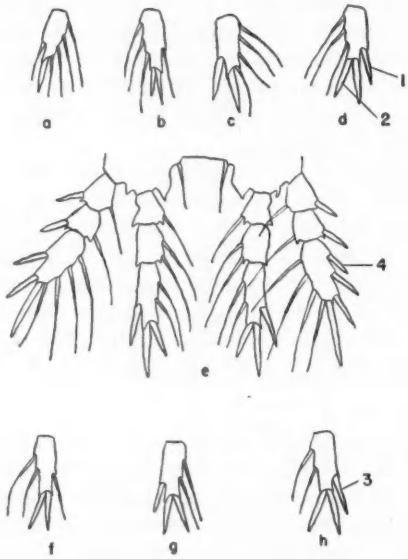

FIG. 3. Swimming leg armature of *C. vernalis* isolates: *a–d*, terminal segment of endopodite of third leg; *a*, isolate A female; *b*, isolate A male; *c*, isolate B female; *d*, isolate C female; *e*, asymmetrical pair of fourth legs of isolate D; *f–h*, terminal segment of endopodite of fourth leg; *f*, isolate A; *g*, isolate B; *h*, isolate C. 1–4 indicate the four loci of variation. (Traced from photomicrographs.)

examined the proximal segment of the endopodite bore one seta, the middle segment two setae, medially. In nearly all specimens the proximal and middle segments of the exopodite bore a spine laterally and a seta medially. Figure 3*e* shows these constant features of the swimming leg armature. No variation occurred in the armature of distal endopodite segments of legs 1 and 2. These segments bore four setae medially, one terminal spine, and one seta laterally.

Variation was found, however, in the armature of terminal endopodite segments of legs 3 and 4 and of all terminal exopodite segments. The structures present at four loci, shown in Fig. 3, were found to vary. Locus 1 is the lateral spine or seta on the terminal endopodite segment of leg 3. Locus 2 is the seta or spine on the distal-medial corner of the same segment. Locus 3 is the position on the fourth leg comparable to locus 1 on the third leg, the lateral seta or spine on the terminal endopodite segment. Locus 4 is on the

terminal exopodite segments of all four legs, and the variation here is the presence or absence of a spine. This character is often described in the literature by giving the number of spines on all the exopodite segments: 3,4,4,4 for spine present at locus 4, or 2,3,3,3 for spine absent.

Loci 1 and 3 can conveniently be considered together since they are h mologous positions on the third and fourth legs respectively. The variation at these loci is shown in Table V for each isolate. Isolates A, E, F, and G most frequently had a seta at both loci, while B, C, and D most frequently had a spine at both. Isolate B is apparently the most variable, many individuals having a seta at locus 1 and a spine at locus 3. This isolate also showed the highest percentage of asymmetries, with a spine on one side and a seta on the other in the same pair of legs. Isolate G showed no variation at all; every individual examined had a seta at both loci.

TABLE V

FREQUENCY OF SPINES OR SETAE AT LOCI 1 AND 3, EXPRESSED AS PERCENTAGES. "UNCLASSIFIABLE" REFERS TO CASES OF ASYMMETRY AT EITHER OR BOTH LOCI

	Isolate						
	A	B	C	D	E	F	G
Total number examined	338	233	70	169	67	124	66
Loci 1 and 3 setae (%)	90	15	0	2	97	82	100
Loci 1 and 3 spines (%)	4	45	96	93	0	6	0
Locus 1 seta, locus 3 spine (%)	2	26	0	2	0	6	0
Locus 1 spine, locus 3 seta (%)	1	1	0	1	1	0	0
Unclassifiable (%)	3	13	4	2	2	6	0

The temperature at which development took place had a marked effect on loci 1 and 3 in the variable isolate B. Table VI shows the condition of these loci in individuals reared at 30°, 25°, and 10° C. At the lowest temperature there was no variation; all individuals had a spine at both loci. At 25° and 30°, however, most specimens developed a seta instead of a spine at locus 1, and some developed a seta at locus 3 as well.

Cultures of all seven isolates were raised at 10° C. These showed no variation at loci 1 and 3. Isolates A, E, F, and G had a seta at both loci, and isolates B, C, and D had a spine at both.

TABLE VI

VARIATION AT LOCI 1 AND 3 OF ISOLATE B REARED AT THREE TEMPERATURES

	30° C.	25° C.	10° C.
Total number examined	21	48	43
Loci 1 and 3 setae (%)	5	27	0
Loci 1 and 3 spines (%)	5	4	100
Locus 1 seta, locus 3 spine (%)	71	48	0
Unclassifiable (%)	19	21	0

TABLE VII

VARIATION AT LOCUS 2 IN FEMALES AND MALES OF EACH ISOLATE

Isolate	Females			Males		
	No. examined	Seta (%)	Spine (%)	No. examined	Seta (%)	Spine (%)
A	192	96	4	59	9	91
B	164	90	10	68	16	84
C	53	0	100	32	0	100
D	103	91	9	33	73	27
E	48	100	0	21	10	90
F	87	100	0	35	11	89
G	45	100	0	19	79	21

In describing variation at locus 2, females and males must be considered separately, since there is apparently a sexual difference here in most of the isolates. The data are presented in Table VII. Females of all the isolates except C predominantly had a seta at locus 2. This was unanimous for isolates E, F, and G. Isolate C is distinctive in having a spine at locus 2 in all individuals examined. Only a few females of A, B, and D had a spine there also. The males of isolates A, B, C, E, and F predominantly had a spine at locus 2, while about three-quarters of those of isolates D and G had a seta there.

The variation at locus 4 is given in Table VIII. All the isolates except G predominantly had the 3,4,4,4, type of armature. Most specimens of isolate G were of the 2,3,3,3 type. Again isolate C was the least variable. The other isolates showed frequent variants which would not fit into either category.

TABLE VIII

VARIATION AT LOCUS 4, EXPRESSED AS % OF INDIVIDUALS HAVING OR LACKING THE SPINES. "UNCLASSIFIABLE" REFERS TO CASES OF ASYMMETRY IN ONE OR MORE PAIRS OF LEGS, OR WHERE LOCUS 4 VARIED FROM ONE PAIR OF LEGS TO ANOTHER IN THE SAME INDIVIDUAL

Isolate	No. examined	Spine present (3,4,4,4) (%)	Spine absent (2,3,3,3) (%)	Unclassifiable (%)
A	342	61	23	16
B	267	73	9	18
C	70	97	0	3
D	171	49	23	28
E	67	52	16	31
F	124	59	22	19
G	66	3	71	26

It is evident that the variation at these four positions is of limited value in identifying the isolates. Yet when the most frequent variant of each isolate is considered, differences between the isolates are apparent. The isolates sort themselves into four groups:

1. Spines at all four loci in both sexes ..... Isolate C
2. Spines at loci 1 and 3, seta at locus 2 in females, spines present at locus 4 ..... Isolates B and D
3. Setae at loci 1 and 3, spine present at locus 4 ..... Isolates A, E, and F
4. Setae at loci 1, 2, and 3, spine at locus 4 absent ..... Isolate G

A character on the fourth legs has been found which distinguishes the otherwise similar isolates B and D. It is illustrated in Fig. 3, and consists of the relative lengths of the two spines terminating the endopodite. In isolate B these spines are approximately equal in length while in D the outer spine is appreciably longer than the inner.

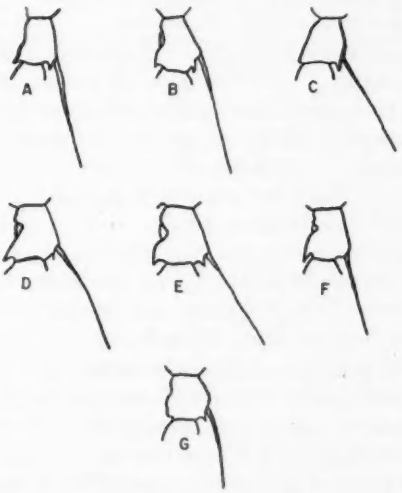

FIG. 4. Basal segment of endopodite of fourth legs of females of isolates A–G. (Traced from photomicrographs.)

Another character on the fourth legs is to some extent useful in separating the isolates. This is the shape of the basal segment of the endopodite of females. Examples are illustrated in Fig. 4. Isolate C is clearly distinguishable from the rest in lacking the notch in the lateral margin shown to various degrees by the other isolates. Males of all seven isolates are alike in lacking the notch. Females of the isolates excepting C can be seen to vary in the prominence and shape of the spur projecting below the notch. Isolates A, B, F, and G are variable but have relatively small spurs, while in isolate D the spur is prominent and projects outward with a straight outer margin, and in E the spur is again prominent but has a curved outer margin.

Anomalies of Swimming Leg Armature

A few individuals of each isolate displayed variations, which from their rarity, might be termed anomalies of swimming leg armature. These are not significant taxonomically but show the extremes to which variation may attain.

The most frequent anomalies constitute the "unclassifiable" fractions in Tables V to VIII. These consisted mostly of asymmetrical patterns in which one or more pairs of legs would bear a spine on one leg where there was a seta on the other, or in the case of locus 4, a spine present on one leg and lacking on the other. An example of this type of asymmetry is shown in Fig. 3. In locus 4, there were also symmetrical deviations from the usual 3,4,4,4 or 2,3,3,3 patterns, such as 3,4,4,3 or 2,3,3,4.

Examples of the complete lack of a spine or seta were observed where instead of the usual structure there was a smooth space. One individual of isolate B had nothing at locus 1 on one side, and one of isolate C had neither a spine nor a seta at locus 3, again only on one side.

Other anomalies concerned loci other than the four discussed in detail. A few individuals of isolates A and B were found to have the structure on the medial margins of the terminal endopodite and exopodite segments of legs 3 and 4, which are normally all setae, partially or completely modified into spines. One individual of isolate B had six spines in place of the usual three or four on the terminal exopodite segment of one leg 4. The other leg 4 and all the other legs were completely normal. One individual of isolate D was found with two spines instead of the usual one on the middle segment of the exopodite of leg 4, and one of isolate A with the same condition in leg 3. A remarkable individual of isolate E had a seta instead of the inner of the two spines on the end of the endopodite of leg 4.

In a few instances a structure could not be positively identified as a spine or a seta. The usual intermediate resembled a seta at the base, but at a variable distance from the base the hairs were replaced by denticles and the margins thickened. One individual of isolate A was found in which a different sort of intermediate occurred, namely an apparent lateral fusion of the terminal spine and the terminal seta on the exopodite of leg 3. This structure had hairs along the inner side and denticles along the outer.

These anomalies illustrate three types of phenomena which may be termed autonomous development of legs, proliferation of parts, and spinification of setae. First, it is apparent that each leg is capable of developing along lines independent of the opposite member of its pair, and of the legs of the other pairs. Secondly, additions to the most frequent formula can occur as well as subtractions. If only occasional losses of parts occurred, mutilation at some stage in development could be suspected. Thirdly, there is apparently a process of spinification of setae which can occur, but usually does not. Usual setae sometimes appear to be replaced by spines, or by some intermediate structure, but seldom is the replacement of a usual spine by a seta observed.

Furcal Setae

On the ends of the furcal rami of Cyclopidae there are four setae,* the middle two very long, the innermost and outermost much shorter. The relative lengths of the inner and outer setae serve as a taxonomic character in some of

*There is also a fifth seta on the dorsal surface near the distal end of the ramus.

the species. In most of the *C. vernalis* isolates the inner seta is about half as long again as the outer. In isolate E, however, these setae are almost equal in length. Table IX shows the mean ratio of their lengths and the range of values for a sample of each isolate. The ratio of inner seta divided by outer is 1.4 to 1.7 for the other isolates and only 1.1 for isolate E.

TABLE IX

RELATIVE LENGTHS OF FURCAL SETAE OF FEMALES, EXPRESSED AS INNERMOST DIVIDED BY OUTERMOST

Isolate	No. of measurements	Ratio of innermost/outermost furcal setae		
		Minimum	Maximum	Mean
A	31	1.2	2.0	1.5
B	8	1.3	1.7	1.5
C	4	1.6	1.9	1.7
D	29	1.3	1.7	1.5
E	9	1.0	1.2	1.1
F	12	1.4	2.2	1.6
G	7	1.2	1.5	1.4

Total Body Length

Cyclopidae, like most arthropods, grow to a finite size at which they remain after reaching maturity. Casual observation indicated that certain isolates were characteristically larger than others. Accordingly, a series of measurements were made on specimens preserved in 70% alcohol to reveal any differences in size between the isolates. Temperature experience during development has a marked effect on final size of Cyclopidae; therefore it is necessary to compare groups raised at known and constant temperatures. Males and females must also be considered separately, because of the characteristic difference in their sizes.

Table X lists the mean lengths of males and females raised at 25° and 10° C., and the ratio of length of 10° specimens to length of 25° specimens for each isolate. In all isolates females were larger than males and both sexes grew to a larger size at the lower temperature. It is interesting that, in females of the five isolates for which data are available, temperature exerted about the same effect on final size. The ratio varied only from 1.22 to 1.28. At 25° extremes of mean length are shown by females of isolates A and C at 1.2 mm. and D at 1.4 mm. At 10° females of isolates A and D were smallest, measuring about 1.5 mm. and of B and F were largest, about 1.7 mm. At 25° males of A and C were smallest, and of F and G largest. At 10° those of A and D were small and B, F, and G larger, but the data are not complete. The greatest differences between isolates amount to about 10% in most cases, not enough for a diagnostic aid, but sufficient to indicate a physiological difference between the isolates. The fact that one isolate, A, appears to be characteristically the smallest, regardless of temperature or sex, also lends significance to the results.

TABLE X

TOTAL LENGTHS IN MM. OF *C. vernalis* ISOLATES REARED AT 10° AND 25° C., AND THE RATIO OF THE LENGTHS AT THESE TEMPERATURES

Isolate	25° C.		10° C.		Ratio
	No. examined	Mean length, mm.	No. examined	Mean length, mm.	Length 10° / Length 25°
Females					
A	23	1.24	4	1.55	1.25
B	19	1.33	9	1.71	1.28
C	18	1.24	–	–	–
D	6	1.40	1	1.53	–
E	4	1.35	8	1.66	1.23
F	16	1.36	3	1.73	1.27
G	7	1.29	6	1.58	1.22
Males					
A	40	0.80	4	1.00	1.25
B	29	0.88	14	1.19	1.35
C	20	0.83	–	–	–
D	11	0.88	3	0.95	1.08
E	–	–	4	1.12	–
F	7	0.95	–	–	–
G	16	0.99	4	1.14	1.15

A more useful size index is afforded by a comparison of the lengths of male and female siblings with identical temperature experience. Again casual observation indicated that certain isolates had relatively small males and others relatively large males in comparison with sibling females. Accordingly the ratio of female length to male length was calculated for every brood whose members were raised at the same temperature and in which the final lengths of adults were measured. The over-all average for each isolate is given in Table XI along with the number of measurements on which each average is based. It can be seen that isolate G had the relatively largest males and D the smallest. For the isolates excepting G, the females were 1.45 to 1.60 times the length of the males, or about half as long again. In isolate G the ratio was only 1.34, or females were about a third as long again as males. It is possible to distinguish G on this index.

TABLE XI

MEAN RELATIVE LENGTHS OF SIBLING MALES AND FEMALES WITH IDENTICAL TEMPERATURE EXPERIENCE

Isolate	No. of males measured	No. of females measured	Mean ratio, female/male
A	53	38	1.54
B	45	32	1.49
C	24	21	1.57
D	17	13	1.60
E	20	22	1.45
F	25	34	1.49
G	25	20	1.34

Key to the Isolates

For the analysis of swimming leg armature, examinations were made of more than 1000 individuals of *Cyclops vernalis* which had been assigned through breeding experiments to seven reproductive isolates. Although variation was found in most of the isolates resulting in considerable overlapping, it was possible to detect differences between the isolates when a large enough number of individuals of each was examined to determine the most frequent variant. Also it was found possible to reduce variation within isolates to almost nil by raising them at a low temperature and the resulting variant corresponded with that which was most frequent at any temperature, except in the extremely variable isolate B.

With the aid of additional characters it is possible to draw up a taxonomic key to the isolates. The key which follows must be considered to apply to the most frequent variant of each isolate, or to the variant obtained at 10° C.

1. Terminal endopodite segment of leg 4 with three spines; terminal endopodite segment of leg 3 with three spines in both sexes; proximal endopodite segment of leg 4 lacking notch in lateral margin in female.....Isolate C
   Terminal endopodite segment of leg 4 with two or three spines; terminal endopodite segment of leg 3 with one or two spines in female, two or three spines in male; proximal endopodite segment of leg 4 having notch or some irregularity in lateral margin in female.....2
2. Terminal endopodite segment of leg 4 generally having only two spines; terminal endopodite segment of leg 3 generally having one spine in female, two spines in male..3
   Terminal endopodite segment of leg 4 generally having three spines; terminal endopodite segment of leg 3 having one or two spines in female, two or three spines in male..6
3. Inner and outer setae of furcal ramus nearly equal in length, outer:inner 1:1.0–1.2; male relatively large, length of female:length of male 1.4–1.5:1.....Isolate E
   Inner seta of furcal ramus much longer than outer, outer:inner 1:1.4–2.0.....4
4. Terminal exopodite segment of legs 1–4 bearing 2,3,3,3 spines respectively in most individuals; male relatively very large, female:male 1.3–1.4:1.....Isolate G
   Terminal exopodite segment of legs 1–4 usually bearing 3,4,4,4 spines respectively; length of female:length of male 1.4–1.6:1.....5
5. Male relatively large, female:male 1.4:1; spur on proximal endopodite segment of leg 4 prominent.....Isolate F
   Male relatively very small, female:male 1.5–1.6:1; spur on proximal endopodite segment of leg 4 small.....Isolate A
6. Terminal spines on terminal endopodite segment of leg 4 about equal in length.....Isolate B
   Outer terminal spine on terminal endopodite segment of leg 4 longer than inner.....Isolate D

The Isolates as Hosts of *Triaenophorus*

In an experiment on host specificity in the tapeworm genus *Triaenophorus*, the seven reproductive isolates of *C. vernalis* were assayed as first intermediate hosts of *Triaenophorus nodulosus* (Pallas) and *T. crassus* Forel.

In the life history of these cestodes, the eggs which are laid by the adult tapeworms hatch into free-swimming larvae known as coracidia. These are eaten by the first intermediate host, a member of the Cyclopidae, where they metamorphose into procercoids in the haemocoele of the host. The infected Cyclopidae are eaten by the second intermediate host, a fish, which is in turn eaten by the final host, the pike, *Esox lucius* Linnaeus.

TABLE XII

RESULTS OF ATTEMPTED INFECTIONS OF *C. vernalis* ISOLATES WITH TWO SPECIES OF *Triaenophorus*

Isolate	No. of individuals exposed to:		% infected by:	
	T. nodulosus	*T. crassus*	*T. nodulosus*	*T. crassus*
A	47	46	11	7
B	19	20	16	0
C	11	10	27	0
D	55	31	4	0
E	22	16	73	75
F	27	35	30	29
G	55	46	64	48

Samples of the seven isolates were fed coracidia of the two species of *Triaenophorus* and examined for procercoids at 1 and 2 weeks after this inoculation. Throughout the experiment they were maintained at 10° C.

The isolates infected by each species of *Triaenophorus* are shown in Table XII. *T. nodulosus* infected all seven isolates, while *T. crassus* infected A, E, F, and G, but not B, C, and D. Isolates E and G appeared to be the best hosts for both species of *Trianeophorus*.

This is a demonstration of the value of an awareness of cryptic speciation. There is an evident difference between the isolates in their suitability as hosts for *Triaenophorus*. It is probable that only four of the seven isolates can function as hosts for *T. crassus*. It can be seen how easily discordant results could be obtained when "*Cyclops vernalis*" as a single species is assayed as a possible host of *T. crassus*. For instance, an investigator using isolate D would achieve remarkably different results from one using isolate E.

Discussion and Conclusions

It has been demonstrated that a pattern of reproductive isolation exists between 30 populations of a widespread and variable cyclopid species, *Cyclops vernalis* Fischer. This pattern suggests the existence of seven reproductive isolates. The breeding experiments from which these conclusions are drawn are convincing because of the high proportion of successes among the controls, and the emergence of an interpretable pattern. The nature of this reproductive isolation is not obvious from observations made during the course of the experiments but seems to be involved in the mechanism of egg extrusion in the female. It was observed that males of one isolate would copulate with and affix spermatophores to females of another isolate, at least occasionally. Yet females of non-conspecific pairs usually did not extrude eggs, even infertile ones, into the external ovisacs. Rather, the eggs were retained in the cephalothoracic oviducts. On the other hand, females of conspecific pairs usually produced external egg masses by the third day after the pair was brought together. The difference in behavior was very marked, therefore, between conspecific and non-conspecific pairs.

Treating the isolates as species, differences between them in morphology, ecology, and host suitability for *Triaenophorus* were sought.

Most of the isolates displayed morphological variability, but the degree and nature of this variability was found to differ from one isolate to another. One isolate (C) scarcely varied at all, and, at the opposite extreme, another (B) was found to vary a great deal. Certain morphological characters were found, however, to distinguish some of the isolates, and a key to their identification could be erected. The fact that all the isolates did not exhibit the same degree and kind of variation is taken as evidence that speciation, albeit of a not easily detected or "cryptic" sort, has occurred in *C. vernalis*.

Ecology of the isolates serves primarily to distinguish one isolate (C) from all the rest. This isolate is found in the plankton, that is, in net tows made in open water, away from substrates of any kind. The difference in behavior is that copepodites of this isolate maintain a free-swimming attitude, while the others tend to crawl about on surfaces.

There appear to be differences between the isolates in temperature tolerance. In laboratory experiments designed to determine the effect of temperature on swimming leg armature and final size, cultures of all seven isolates were raised at 10°, 25°, and 30° C. All isolates lived at 10°, at 25° most cultures of isolate E died out, and at 30° most of C, D, E, and G died out. Lethal temperatures should be investigated for each isolate, but from this evidence it would appear that A, B, and F tolerate the highest temperatures, C, D, and G are next, and E has the lowest temperature tolerance.

It was also demonstrated that the isolates differed in their ability to become parasitized by *Triaenophorus*. This fact has not much taxonomic significance since many other unrelated species of Cyclopidae are also infectible. It is a demonstration, though, of the usefulness of an accurate taxonomy.

Morphological variability of *C. vernalis* has led to a complex and variable nomenclature. It might be well to describe briefly the changes of thought on this species or group in North America.

Originally *Cyclops viridis* Jurine was considered to be the basic species and morphological variants of it were assigned to subspecific rank by Marsh (1910 (9)). He named *C. viridis* var. *americanus* and relegated Herrick's earlier (1895 (5)) species names *parcus* and *brevispinosus* to two more varieties of *C. viridis*. The principal distinguishing characters for these varieties were given as the numbers of spines on the terminal segments of both rami of the fourth legs. *C. v. americanus* Marsh had two spines on the terminal endopodite segment and four on the terminal exopodite segment, like isolates A, E, and F of the present study and some variants of B. *C. v. parcus* Herrick also had two spines on the endopodite, but had only three spines on the exopodite, like G of the present study, and some variants of A. *C. v. brevispinosus* Herrick had three spines on the endopodite and four on the exopodite, like isolates B, C, and D. However Herrick (1895 (5)) gave other characters of his *C. brevispinosus* which definitely indicate isolate C. We shall return to this species presently.

Gurney (1933 (4)) pointed out that American Cyclopidae of the so-called *viridis* group resembled *Cyclops vernalis* Fischer rather than *C. viridis* and listed differences between the two species. He therefore assigned Marsh's and Herrick's varieties to *C. vernalis*. Coker (1934 (2)) tried unsuccessfully to cross European *C. viridis* with American *C. vernalis*, showing that they are truly separate species. Yeatman (1944 (13)) reiterated the distinctions between the two species and made reference to the *vernalis* group. The true *C. viridis* does not enter into the present work, and may not even occur in North America, so we may restrict further discussion to the *vernalis* group.

The only indication other than gross morphological variation that the American *vernalis* group might contain more than one species came with the chromosome work of Chambers (1912 (1)). He found that Cyclopidae which he called *C. americanus*, *C. parcus*, and *C. brevispinosus* had different diploid chromosome numbers, namely 10, 6, and 4 respectively. It is interesting that he described two "forms" of *C. brevispinosus* which he called *parcus* form and *americanus* form. Both had three spines on the endopodite of leg 4 but the former had only three spines on the terminal exopodite segment where the latter had four. In the present study, a frequent variant of isolate D would correspond to Chambers' *C. brevispinosus*, *parcus* form.

The tendency prevalent in the recent American literature has been to consider the *vernalis* group as a single species *C. vernalis* and to relegate the names *C. americanus*, *C. parcus*, and *C. brevispinosus* to its synonymy. Yeatman (1944 (13)) has done this on evidence that he and others had raised individuals identical with these three types from single broods of *C. vernalis*. The present study has shown that this could be done with isolate B, which is particularly variable. Pennak (1953 (11)) and Yeatman (unpublished manuscript) do not split the species *C. vernalis*. They could not do otherwise on the existing evidence.

The names *C. americanus* and *C. parcus* seem to have no definite application to the isolates, based as they are on the characters of swimming leg armature which vary the most. The name *C. brevispinosus*, on the other hand, probably refers to isolate C of the present study.

Herrick and Turner (1895 (5)) described and figured *C. brevispinosus* as having spines at the four positions we have called loci 1 to 4 and as lacking any notch or undulation in the lateral side of the proximal segment of the fourth leg. The species was reported to be planktonic by these authors and by Marsh (1903 (8) and 1910 (9)). The present study has shown that only one isolate out of the seven was characteristically planktonic and lacked the notch in the basal endopodite segment of leg 4. This isolate was also the only one invariably having the spine–seta formula of swimming legs identical with that of *C. brevispinosus*, although in four of the other isolates this formula occurred in a few individuals. The author is in favor of reinstating Herrick's species name and applying it to isolate C.

No attempt will be made to name the other isolates. For one reason, the number of such isolates is not known. Breeding experiments performed on

more collections from widely distributed places may reveal several more isolates, each with its own scope for morphological variation. The small degree of difference between the isolates in outward appearance and the amount of intraspecific variation with overlapping makes it impossible to identify all of the already discovered isolates without breeding tests. Unique characters can identify D and E, but A, B, F, and G have some indistinguishable variants. It is necessary to perform breeding experiments to identify new collections or to determine the type of variation and the most frequent variant by examining large numbers of individuals. The former must be considered the more reliable procedure.

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PECTINASE IN CERTAIN INSECTS[1]

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Abstract

Pectinase was found in the saliva of 23 species of aphids, one species of leafhopper, and one species of adelgid. The enzyme was not found in four species of aphids in their apterous form, and one species of psyllid, or in the saliva of the alate form of five species of aphids that contained pectinase in the apterous form. These results emphasize the need to consider each form of a species of aphid individually. The discussion considers similarities in the mode of tissue penetration by fungi and insects, and briefly the possibility that cell wall digestion by pectinase may facilitate the extraction of virus particles by insect vectors.

Introduction

In 1951, Kertesz (7) stated that "in animals the presence of pectin-polygalacturonase has been only shown in snails". McAllan and Cameron (9) and Adams and McAllan (1) have since demonstrated its presence in extracts of the aphids *Myzus persicae* (Sulz.), *Macrosiphum solanifolii* (Ashm.), *Aulacorthum solani* (Kltb.), and *Aphis abbreviata* Patch and in the salivary secretion of *M. persicae*. The present investigation was undertaken to study its occurrence in some other aphid species and in some other insects with piercing–sucking mouth parts.

Methods

The method previously used for the determination of pectinase in the saliva of *M. persicae* (1) provides a simple and sensitive test for the presence of pectinase in other insects that attempt to feed in a similar manner. This test is based on observations that aphids probe surfaces readily and release droplets of saliva that, if they contain pectinase, hydrolyze pectic acid applied to filter paper. As negative results with this technique do not prove the absence of pectinase, some insects yielding negative results in the salivary tests were examined further for enzyme activity by adding extracts of whole specimens to pectic acid solutions. The latter technique gave positive results with concentrations of pectinase to 10^{-7} g./ml.

The tests were conducted by caging 15–30 insects over spots of 1% pectic acid solution on filter paper, or by incubating extracts of 200–300 insects in acetate buffer, pH 4.6, with 1% pectic acid. The presence of the products of the enzymatic hydrolysis of pectic acid was demonstrated chromatographically by using the upper layer of a 4:1:5 mixture of *n*-butanol, acetic acid, and water, by spraying the chromatogram with an aniline acid phthalate solution, and by heating it at 105° C. for 2–3 minutes (9). Controls consisting

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of pectic acid alone, insect extract alone, insect saliva from probing alone, and frass and honeydew were examined separately. The aphids were collected locally as they became available in the field.

Results and Discussion

Table I indicates that most adult apterous insects examined contain pectinase. In some aphids the alate form differed from the apterous form of the same species from the same host by lacking detectable pectinase. Sometimes the same species and form of aphid gave positive results on one host but negative results on another. Where both extracts of whole insects and saliva from probing were examined in the same form and species, the results were similar.

The table also shows the host plant from which test material was obtained and indicates the season by date. Generally apterous forms of aphids contained pectinase when examined from plants normally referred to in the literature as their secondary hosts. An apparent exception to this was *Phorodon humuli* from *Humulus*. When pectinase was found in the alate form, it was always present in the apterous form from the same host. The reverse was not true, however; in many cases in which apterous forms contained pectinase, alate forms from the same host lacked it.

The results of this study support the concept of two kinds of alate aphid forms, which have been designated "migrant" and "dispersal" in earlier literature (12). These terms imply a change from succulent summer hosts to more persistent perennials or biennials in the case of "migrants", and a spreading to similar hosts in the case of "dispersals". Patch (11) showed that the migrant form of *Prociphilus tessellata* does not feed. If this is generally so in other migrants, the lack of pectinase in "migrants" is not surprising.

That pectinase was found in the apterous but not in the alate forms of certain aphids is further support for MacGillivray's contention (8) that aphids may not be considered in a general sense, but rather that even forms of the same species are distinct animals among which manifold and perhaps important physiological differences occur.

There are other examples of differences in enzyme content between forms of the same insect, and between bugs feeding on different hosts (Nuorteva (10), Baptist (2), (Duspiva (5 and 6)).

In literature reviewed for this study similarities were noted repeatedly between the methods of penetration of plant tissue by parasitic fungi and the methods used by aphids. Brown (3) summarized recent work on the physiology of parasitism and demonstrated the association of polygalacturonase with several plant-invading parasites. This enzyme, secreted by the tips of advancing mycelia, assists fungi to penetrate tissue by breaking down the pectin of the middle lamellae. Since intercellular penetration of tissues along the middle lamella seems general among aphids (4), it seems reasonable to expect the same result from the secretion of the same enzyme by advancing stylets.

TABLE I

PECTINASE ACTIVITY IN THE SALIVARY SECRETIONS AND EXTRACTS OF SOME APHIDS AND OTHER INSECTS

Species	Host	Date	Reaction			
			Secretion		Extract	
			Apt.	Al.	Apt.	Al.
Aphididae						
Aphis abbreviata Patch	*Solanum tuberosum* L.	3/1/55			+	
Aphis abbreviata Patch	*Solanum tuberosum* L.	15/7/56	+	+		
Aphis abbreviata Patch	*Rhamnus* sp.	26/6/57	+			
Aphis abbreviata Patch	*Rorippa* sp.	16/8/57	+	+		
Aphis abbreviata Patch	*Rumex* sp.	16/8/57	+			
Aphis cardui L.	*Prunus* sp.	13/6/55			+	
Aphis fabae Scopoli	*Tropaeolum* sp.	15/7/56	+	−		
Aphis fabae Scopoli	*Tropaeolum* sp.	16/8/57	+	+		
Aphis fabae Scopoli	*Syringa* sp.	22/7/57	+			
Aphis pomi DeGeer	*Malus* sp.	19/6/56	+			
Aphis pomi DeGeer	*Malus* sp.	10/7/57	+	+		
Aphis sedi Kltb.	*Sedum* sp.	24/7/56	+	−		−
Aphis spiraecola Patch	*Celastris* sp.	17/7/56	+			
Aphis spiraecola Patch	*Spirea* sp.	19/7/56	+	−		−
Aphis spiraecola Patch	*Spirea* sp.	10/7/57	−	−		
Aulacorthum solani	*S. tuberosum* L.	−/9/55			+	
Aulacorthum solani	*S. tuberosum* L.	18/6/56	+			
Ceruraphis viburnicola (Gill.)	*Viburnum opulus* L.	−/9/55			+	
Ceruraphis viburnicola (Gill.)	*Viburnum opulus* L.	14/6/57		+(im.)*		
Ceruraphis viburnicola (Gill.)	*Carex* sp.	10/7/57	−			
Ceruraphis eriophori (Wlk.)	*Viburnum opulus* L.	14/6/57		−		
Ceruraphis eriophori (Wlk.)	*Carex* sp.	10/7/57	−			
Cinara curvipes Patch	*Abies balsamea* L.	13/9/56				
Cinara sp.	*Pinus strobus* L.	10/7/57	+			
Dactynotus sp.	*Solidago* sp.	16/7/56	+	+		
Dactynotus sp.	*Rudbeckia laciniata* L.	8/8/57	+	+		
Eriosoma americanum (Riley)	*Ulmus americana* L.	9/7/56	+	−		
Eriosoma lanigerum (Hausm.)	*Ulmus americana* L. (rosetted)	2/7/57	+	+(im.)		
Eriosoma lanigerum (Hausm.)	*Ulmus americana* L. (curled)	9/7/56	+	−		
Rhopalosiphum maidis (Fitch)	*Hordeum* sp.	1/8/56	−	−		
Rhopalosiphum maidis (Fitch)	*Zea mays* L.	6/9/56	−			
(*Rhopalosiphum maidis*)	*Sedum* sp.	17/8/57		+		
Rhopalosiphum rhois (Monell)	*Rhus typhina* L.	16/7/57	+	+		
Schizolachnus pini-radiata (Davidson)	*Pinus resinosa* Ait.	10/7/57	+			
Thomasiniellula populicola (Thomas)	*Populus* sp.	26/7/56	+			
Adelgidae						
Adelges piceae (Ratz.)	*Piceae* sp.	30/8/55			+	
Cicadellidae						
Dalbulus maidis (Del and Wohl.)	*Zea mays* L.	3/7/56	+(adult)			
Lygaeidae						
Liocoris lineolaris (Beauv.)	*S. tuberosum* L.	28/6/56	+(adult)			
Psyllidae						
Unidentified	*Juglans* sp.	28/6/56	−(nymphal)		−	
Orthoptera						
Melanoplus femur-rubrum (DeGeer)	*Trifolium* sp.	−/9/54				−(adult)

*(im.) = wing-pad form, immature. Apt. = apterous. Al. = alate.

What functions salivary enzymes might serve are speculative. Pectinase may aid in penetration of tissues (1), the end-products of the hydrolysis of pectin might contribute to the nutritional needs of the insects, or digestion of cell walls might well facilitate the extraction of virus particles by their insect vectors.

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SOME ASPECTS OF THE EPIDEMIOLOGY OF A MICROSPORIDIAN PARASITE OF THE SPRUCE BUDWORM, CHORISTONEURA FUMIFERANA (CLEM.)[1]

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Abstract

Limited tests indicate that the microsporidian parasite *Perezia fumiferanae* is restricted to insect hosts of the genus *Choristoneura* and for practical purposes to the single species *Choristoneura fumiferana* (Clem.). Frozen spores of this parasite were found to be infectious longer than those kept under other conditions but no spores were infectious after 6 months' storage. There are two distinct methods of infection, oral and congenital. Immature eggs within infected female insects are infected by schizonts which develop into spores after the eggs are laid. All infected females, regardless of the degree of infection, transmit the parasite to their offspring, and for practical purposes all the progeny of such females are infected. Offspring of heavily infected females appear to contain more spores than those of lightly infected females. Infected males are sometimes capable of transmitting the parasite to a portion of their offspring. Congenital transmission is responsible for the passage of the parasite from host generation to generation. Increase in the incidence of the parasite occurs by oral ingestion of spores but the habits of the host larvae restrict most transmission to the late larval instars.

Introduction

The presence of a microsporidian parasite in spruce budworm populations in Ontario was first noted in 1949 (2). In 1955 the life cycle of the parasite was described and it was named *Perezia fumiferanae* Thom. (8). The present paper is concerned with some of the inherent characteristics of the host and of the parasite which influence the incidence of the parasite in budworm populations. Much of the material used in this paper was gathered from the Uxbridge Forest budworm population, near Uxbridge, Ont., which was approximately 40% infected by *P. fumiferanae*.

Spore Longevity

As the spore is an infective stage of the parasite, its longevity is an important epidemiological factor. To determine spore longevity under various conditions an aqueous spore suspension containing 20×10^6 spores per cc. was prepared from larvae recently killed by the parasite. This was divided into 21 parts of 1 cc. each: 7 portions were dried in beakers at room temperature; 7 were maintained at $+5°$ C. in the refrigerator; and the remaining 7 were frozen at $-5°$ C. At intervals, samples from each of these groups were fed to larvae, which were examined after 2 weeks for infection. Uninfected larvae were also reared as controls. In addition, whole larvae that had been

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TABLE I

PERCENTAGE* OF LARVAE INFECTED BY SPORES KEPT UNDER SEVERAL CONDITIONS FOR VARYING LENGTHS OF TIME

Condition of storage	Percentage of larvae infected by spores treated for:							
	1	2	4	6	10	16	24	32 weeks
Dry, 20° C.	90	80	20	0	0	0	0	–
Wet, 5° C.	80	80	80	20	0	0	0	–
Frozen, −5° C.	90	60	100	80	90	80	0	–
Frozen in insect	–	–	–	–	–	–	–	0
Control (no spores)	10	0	0	0	0	0	0	0

*Ten insects in each group.

killed by the disease were frozen at −5° C. for 8 months, the spores were then extracted and fed to larvae. The results of these experiments are shown in Table I.

It is quite apparent from these results that the spores do not have a long life. Drying seems to be especially unfavorable for spore longevity, most of the spores dying from two to four weeks after being dried. Frozen spores remained viable longer than those under other conditions, being infective after 16 weeks' storage. Although cold aqueous suspensions of spores lost most of their viability after four weeks' storage, spores stored in this manner have occasionally been observed to be viable for periods of from 12 to 16 weeks. It is believed that the degree and nature of bacterial contamination of these suspensions may play an important part in determining their viability. Spores preserved in frozen insect cadavers for 8 months were not viable.

The most important implication of this experiment is that the spores cannot last from year to year in the external environment of the insect. As there is a 9-month dormant period during the second instar and the spores have not been observed to remain viable for more than four months, it is apparent that the parasite must be carried over this dormant period within the insect. Thus, each spring the initial infection in the field must be attributed to the emerging insects and not to casual contamination of the environment by the preceding generation.

Host Specificity

Host specificity is an important epidemiological factor. If an obligate parasite is restricted to one host species, its survival is intimately connected with that of the host, and any factor, including the parasite, exerting an unfavorable influence on the host will also adversely affect the parasite. On the other hand, a parasite that can infect more than one host has greatly enhanced chances of survival and spread.

The host specificity of *P. fumiferanae* was tested against the following insects:

Choristoneura pinus Free.	(Lepidoptera–Tortricidae)
Acleris variana (Fern.)	(Lepidoptera–Tortricidae)
Archips cerasivorana (Fitch)	(Lepidoptera–Tortricidae)
Malacosoma disstria Hbn.	(Lepidoptera–Lasiocampidae)
Apanteles fumiferanae Vier.	(Hymenoptera–Braconidae)
Glypta fumiferanae (Vier.)	(Hymenoptera–Ichneumonidae)

Of these insects, only *C. pinus*, the jack pine budworm, became infected.

Through the courtesy of Dr. J. Franz of the Institut für biologische Schädlingsbekämpfung, Darmstadt, West Germany, *P. fumiferanae* was also tested against *Choristoneura murinana* (Hbn.), a closely related European species. Dr. Franz reported that of 60 test larvae, 26 died: of these two contained spores that were identified as *P. fumiferanae*. None of 60 control larvae contained the microsporidian.

On the basis of these limited tests the parasite appears to be limited to the genus *Choristoneura*. Although *C. pinus* is susceptible to infection under laboratory conditions, it is doubtful whether *C. pinus* populations support the parasite in the field—only one case of a jack pine budworm infected with a microsporidian has appeared in the Insect Disease Survey since its origin in 1952. Whatever the reasons for this, the existence of the parasite is apparently dependent to a very great extent upon the spruce budworm.

Transmission

Under natural conditions budworm larvae become infected with *P. fumiferanae* by the oral ingestion of spores, or congenitally.

Infection per os

The source of spores for oral infection is the diseased insect, either living or dead, and its by-products. Active, infected insects pass spores both in the frass and in the fluid material that they regurgitate when alarmed; as the disease progresses the number of spores passed in these ways increases. Silk and shed integument have also, on occasion, been found to contain spores, as have the fluid wastes excreted by emerging adults. Larvae killed by the disease are very rich sources of infection, containing millions of spores.

Spores from the above sources may be dispersed by various physical factors, such as wind and rain, and biological factors, such as predators and parasites, and occasionally by the budworm itself. Wind and rain are undoubtedly of value in breaking up the bodies of dead insects thus releasing the spores and dispersing them over large areas. Although the importance of parasites and predators has not been assessed, predators may spread the disease by feeding on diseased insects and subsequently passing the spores in their feces. Weiser (9) draws attention to the fact that insects infected by microsporidian parasites are especially easy prey for predators. Hymenopterous and dipterous

parasites, passing from insect to insect, may well carry spores on their bodies; Blunck (3, 4) reported that *Apanteles glomeratus* L. was able to transmit a microsporidian from host to host by means of its contaminated ovipositor. Cannibalism, although fairly common in the laboratory, is not thought to be of frequent occurrence in nature except in the case of sixth-instar larvae feeding on freshly formed pupae. By these means spores may become dispersed in the general environment of the insect to serve as potential sources of infection.

Congenital Transmission

Passage of *Nosema bombycis* through the eggs of the silkworm was reported by Pasteur as early as 1870 (6), and since that time many other workers have reported transovarial transmission of microsporidian diseases of insects. However, according to Steinhaus (7), Masera (5) cast doubt on many of these claims by rearing healthy silkworm from "diseased" eggs after disinfecting the exterior of the eggs. Thus infection of the progeny may sometimes result from contamination of the egg surface rather than infection within the egg.

Congenital infection of budworm larvae by *P. fumiferanae* was first suspected when second-instar larvae in the hibernacula were found to contain spores. As the larvae do not feed until after emergence from the hibernacula, there was little chance of the larvae becoming infected by the parasite from contaminated foliage. First-instar larvae that were killed and fixed immediately after hatching were found to have spores in the yolk material within the gut (Fig. 1). Eggs sectioned at the "black-head" stage (about six days old) were likewise found to have spores in the yolk. Moreover, although spores could not be found in sections of freshly-laid eggs, schizonts could be distinguished among the yolk granules after careful Giemsa-staining. These schizonts could also be found in the immature eggs within the ovarioles (Fig. 2). It is evident that the parasite is passed from infected females to their offspring through the eggs.

As the parasite is suspended, and apparently inactive, in the yolk mass of the egg, the first-instar larvae, and the overwintering period of the second-instar larvae, these stages cannot be truly termed infected. Infection occurs near the time of spring emergence when the spores germinate. Despite the fact that the term "infected" is not technically correct it is used here to denote the presence of the parasite within the host.

It is interesting that transmission is by schizonts and that the spores do not appear until several days after the eggs have been laid. If this is the case with other species it might explain Allen's (1) observation that heat treatment (47° C.) of potato tuberworm eggs to eliminate a microsporidian is much more effective when applied to young eggs rather than eggs about to hatch. It is known that the treatment at this temperature is not lethal to spores. It might also explain Allen's observation that, although eggs from

PLATE I

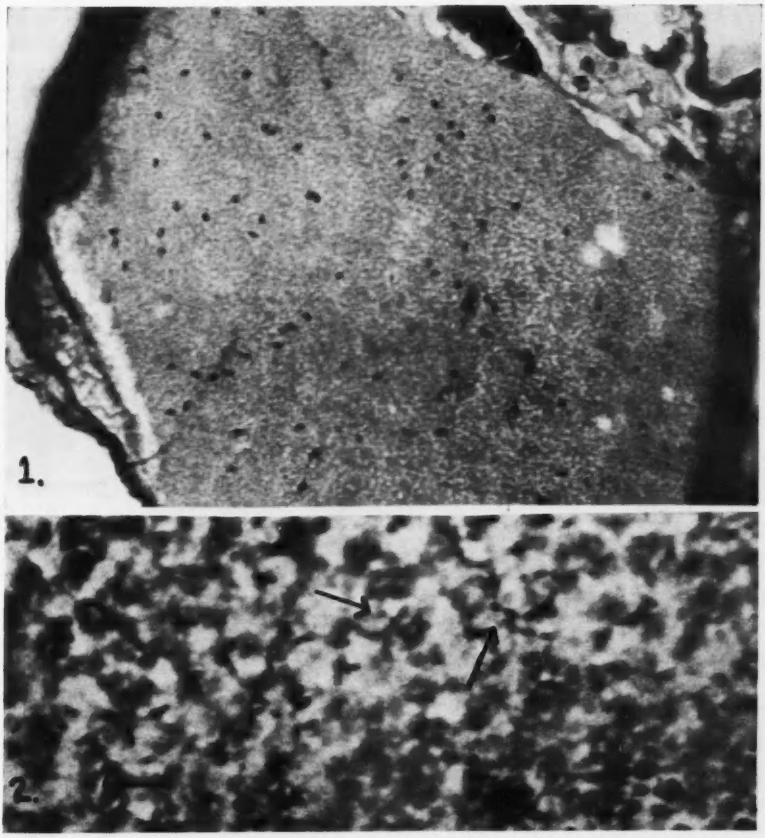

FIG. 1. Spores of *P. fumiferanae* within the yolk body of newly-hatched budworm larvae. Iron hematoxylin. ×470

FIG. 2. Schizonts of *P. fumiferanae* in the yolk of an egg in ovary of infected budworm female. Giemsa stain. ×780

infected individuals have a high incidence of infection, they are not infectious when fed to other larvae. Presumably only spores can survive ingestion; schizonts, being intracellular forms, are probably unable to withstand the conditions occurring within the digestive tract.

Extent of Congenital Transmission

Several experiments were conducted to determine the frequency, variation, and extent of congenital transmission. Samples of the offspring of non-infected, lightly infected, and heavily infected females, all mated with non-infected males were compared to determine the proportion of infected larvae in each group. Five larvae from each mating were examined individually for the presence of the parasite. In all, 20 matings of each of the infected type of females and 10 matings of non-infected females were examined. The results are shown in Table II.

The following conclusions are drawn from the experiment: (*a*) All infected females, regardless of the degree of infection, transmit the parasite to their offspring. (*b*) For practical purposes, all the progeny of infected females are infected. (*c*) Although it does not show in Table II, the offspring of heavily infected females appear to contain more spores than those of lightly infected females.

TABLE II

NUMBER OF DISEASED OFFSPRING IN SAMPLES FROM NON-INFECTED, LIGHTLY INFECTED, AND HEAVILY INFECTED FEMALES MATED WITH NON-INFECTED MALES

Condition of female	No. families	Total no. larvae examined	No. larvae infected
Non-infected	10	50	0
Lightly infected	20	100	100
Heavily infected	20	100	99

To determine whether the male parent is able to transmit the parasite to its progeny, samples of 10 families from healthy female × infected male matings, were examined. The results are presented in Table III. It may be seen that most matings did not give rise to infected larvae, but it is clear that infected males are sometimes capable of transmitting the parasite to some or all of their offspring. This is not surprising as the testes are frequently (although not invariably) infected and spores can be seen lying among the bundles of sperm. Possibly these spores are passed to the female in the spermatophores and stored in the spermatheca. From there they could enter the micropyle of the egg with the sperm or perhaps contaminate the surface of the egg. More has to be learned about the structure and development of the embryonic and young larval stages of the budworm before these processes can be fully elucidated.

TABLE III

TRANSMISSION OF PARASITE FROM MALE PARENT TO OFFSPRING (10 LARVAE EXAMINED/FAMILY)

Family No.	Condition of larvae		% infected
	Non-diseased	Diseased	
1	10	0	0
2	10	0	0
3	0	10	100
4	10	0	0
5	10	0	0
6	5	5	50
7	10	0	0
8	8	2	20
9	10	0	0
10	10	0	0

Source of Infected Adults

Although rearing experiments have shown that some of the congenitally infected larvae survive to the adult stage, most adult infections result from the ingestion of spores during the larval period. Because of the importance of infected adults in the transmission of the parasite from generation to generation, the following experiment was conducted to determine whether larvae, infected late in larval life, would develop into infected adults. Ten sixth-instar larvae were fed an aqueous suspension of spores, the number of days before pupation was noted and the resulting adults were examined for infection. A rough estimate of the degree of infection was made by counting the number of spores appearing in the field of the microscope. A similar number of larvae were reared on clean foliage as controls. The results are shown in Table IV. It is apparent that infection as late as 3 days before pupation will result in infection of the adult. While it is true that the resulting infection is light, such adults can transmit the infection.

TABLE IV

INFECTION OF THE ADULT BY INGESTION OF SPORES DURING LATE LARVAL PERIOD

Larva No.	Days before pupation	Diagnosis of adult
1	3	Infected, light
2	5	medium
3	5	medium
4	6	medium
5	6	medium
6	7	medium-heavy
7	7	medium
8	8	medium-heavy
9	11	heavy
10	11	Died as pupa, heavily infected

NOTE: None of the control larvae was infected.

Effectiveness of Transmission

To determine the variation in the level of infection in succeeding generations, measurements were made on the Uxbridge Forest population over a period of three years.

Foliage from the Uxbridge Forest bearing hibernating larvae was brought to room temperature early each spring. As the larvae emerged they were placed in vials and reared individually. All the insects were examined microscopically for the presence of the disease and the percentage of the population infected was thus determined. The results shown in Table V indicate that under the conditions prevalent in the Uxbridge Forest during these years the parasite was able to increase the level of infection. However, it should be noted that during the test years both the host and the parasite populations levels were high; under less favorable conditions the level of infection might fall.

TABLE V

PERCENTAGE OF OVERWINTERING POPULATION INFECTED BY *P. fumiferanae*

Year	Sample size	No. infected larvae	% larvae infected
1955	104	38	36.5
1956	174	76	43.6
1957	123	69	56.1

Discussion

The data on the incidence of infection in the overwintering Uxbridge Forest population indicate that the parasite is able not only to maintain a given level of infection, but on occasion to increase this level in successive generations. The question arises as to how and when this increase occurs.

The parasite is passed from generation to generation by the extremely efficient method of transovarial transmission. However, as infected females lay fewer eggs than their healthy counterparts* the incidence of infection would tend to decrease in successive generations. Although this decrease may be compensated for by the males that pass the parasite to their offspring, it is questionable whether congenital transmission serves to increase the proportion of infected individuals in the following generation.

It is apparent that any increase in the incidence of the parasite must occur by the dissemination and ingestion of viable spores during the feeding larval period. However, it cannot be assumed that infection occurs at a steady rate throughout this period. As the parasite appears to be restricted to the spruce budworm, and as this insect does not feed from the last larval instar in July until the emerging second instar in May, and as the spore longevity is not sufficient to last in vitro throughout this period, the environment of

*Data on which this statement is based will be published in a forthcoming issue of this journal.

the second-instar larvae emerging in the spring is virtually free of sources of oral infection. However, as the larvae develop, dissemination of spores by the congenitally infected larvae begins. Transmission is not at first effective as the young larvae are largely isolated by their feeding habits. The larvae first excavate old needles and later feed deep within the developing buds and, as they require only small quantities of food, are seldom forced to search for new feeding sites. By the time of the late fourth or early fifth instar some mortality has occurred among the congenitally infected larvae. This mortality greatly increases the quantity of spores in the environment of the host insect and is correlated with a gradual increase in the feeding activity of the host. By the time of the fifth instar most mortality, directly attributable to the parasite, has occurred and the insects are feeding voraciously and as a result are forced to move to new feeding sites almost daily. Thus it is only near the end of the larval period that the insects stand in great risk of infection and the opportunity occurs to increase the proportion of infected individuals in the population. Larvae infected at this time will, for the most part, develop into infected adults and thus ensure transmission of the infection to the following generation.

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ANATOMY AND HISTOLOGY OF THE FULL-GROWN LARVA OF CTENICERA AERIPENNIS DESTRUCTOR (BROWN) (COLEOPTERA : ELATERIDAE)[1]

D. C. EIDT[2]

Abstract

The internal anatomy and histology of the full-grown larva of *Ctenicera aeripennis destructor*, the prairie grain wireworm, is described and illustrated in part. Included are the digestive, circulatory, respiratory, and nervous systems; the fat body, oenocytes, corpora allata; and the muscles of the head, digestive tract, and dorsal vessel.

Ctenicera aeripennis destructor (Brown), the prairie grain wireworm, is a native of the North American prairies, where it feeds on native grasses. As an agricultural community became established it found desirable host plants among those that were being cultivated. It has caused serious damage to the wheat crop from the earliest days of grain farming on the Canadian prairies (34).

Research on control of this species has been conducted at the Saskatoon laboratory on a large scale for many years, and during recent years the importance of fundamental studies has become increasingly apparent. Nutritional studies were begun as part of a program of physiological investigation; behavior and ecological studies are being expanded and intensified. Because a thorough knowledge of the anatomy is important in these investigations, and for its academic interest, this study was undertaken.

The literature contains very little about the internal anatomy of elaterid larvae. James (20) wrote a short account on *Agriotes obscurus* (L)., but he was particularly interested in the "so-called sensory pits of the ninth abdominal segment" and wrote little about other structures. His paper contains a brief description of the digestive system with four figures and brief notes on the Malpighian tubules, central nervous system, dorsal vessel, and fat body.

Four other authors make brief reference to anatomy of elaterid larvae. Paillot (27), in a comparative study, described and illustrated the hemocytes, but did not mention which species he examined. Glen (15), in an account of the external morphology, described the preoral cavity and noted the external appearance of the spiracles of *C. aeripennis destructor*. Snodgrass (32) described the biforate spiracles of *Alaus oculatus* (L.) in detail and pointed out that a thorough, comparative study of biforate spiracles in Coleoptera is needed. Beier (3) published descriptions of the central nervous systems of *A. oculatus* and *Elater sanguineus* L., along with other coleopterous

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Contribution No. 3719, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada; from a thesis submitted to the Faculty of Graduate Studies, University of Saskatchewan, in partial fulfillment of the requirements for the Ph.D. degree.
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larvae, but said little about their histology. He was particularly interested in coalescence in the central nervous system from a phylogenetic standpoint. Dorsey (9), in a study of the labrum, labium, and pharyngeal region of Coleoptera, described and illustrated the labial, cibarial, pharyngeal, and hypopharyngeal muscles of *Parallelostethus attenuatus* (Say) and *Alaus* sp. He noted that labral muscles are absent.

This paper deals with the structure of the various organs and tissues and with the histology of many of them. The histological structure of some, for example, the subesophageal ganglion, is not well known in any insect. Such problems are better studied in comparison with other insects and to do so here is beyond the scope of this paper. For all the postembryonic stages the reproductive system was described by Zacharuk (41).

Materials and Methods

The larvae used in this study were collected in the vicinity of Saskatoon, Sask. The larvae are termed "full-grown" because the exact stadium cannot be determined, and only large larvae were selected. Specimens were stored alive at temperatures slightly above freezing, where they were inactive and protected from cannibalism and fungi.

The larval cuticle is thick and hard, but good sections were obtained when freshly molted specimens were selected. When specimens were required, they were removed to room temperature and placed in salve tins with soil and wheat kernels. They were checked daily and freshly molted specimens were removed. They were then cut in two with scissors and fixed in Bouin's fixative.

Sections were cut from tissue embedded in Tissuemat, and were stained with Delafield's haematoxylin and eosin.

Blood smears were stained with prepared commercial Wright's stain. Air-dried smears were stained for 1 minute after which the stain was diluted about one-half with water and left for 4 minutes. They were then washed in running water, dried, and made permanent with a cover slip secured with euparol.

Numerous dissections were prepared. Paracarmine and Oppel's trichrome stain were used to stain most dissections. Dixon (8) used paracarmine with good results, but he made it with glycerine whereas I made it with alcohol. Paracarmine in alcohol caused some shrinkage, but this was an advantage because it resulted in the separation of parts, such as muscle fascicles, which were not otherwise easily seen. The oenocytes could be demonstrated remarkably well in situ by staining dorsal dissections with haematoxylin. Tracheae were stained with mercurochrome after all soft tissue was removed by digestion with a pepsin – hydrochloric acid solution.

Most of the drawings were prepared by using squared paper and a squared ocular micrometer. They were then transferred to Bristol board and inked. Many of the drawings of lower magnification were traced on Bristol board

from a projected image of the section. The dimensions of the fat cells, blood cells, and oenocytes are given but the scale of magnification of most drawings is not given. Individual variation is considerable, and the magnification would add nothing to the value of most of the observations made.

The terminology used is based on that of Snodgrass (32). Where reference is made to features of the external anatomy, the terminology of Glen (15) is used.

Anatomy and Histology

Digestive System

The digestive tract (Fig. 1) is a simple structure. It is not looped and coiled as in many insects but follows a direct course from the mouth to the anus, as does the digestive tract of *Tenebrio molitor* L. (14). It consists of pharynx, esophagus, mesenteron, anterior intestine, and rectum. Salivary glands, enteric caeca, peritrophic membrane, and rectal pads are absent. These structures are also absent in the larva of *Agriotes obscurus* (20). The absence of salivary glands is not unusual because, according to Snodgrass (32), mandibular and maxillary glands are not always present and labial glands are absent in Coleoptera. Enteric caeca are frequently present in Coleoptera (32), but the peritrophic membrane and rectal pads are not always present. The preoral cavity and Malpighian tubules are considered here although they are not, strictly speaking, parts of the digestive system.

Preoral Cavity

Glen (15) gives an extensive description of the preoral cavity. The mouth, which Glen observed to be small and transverse, marks the true beginning of the stomodaeum. The mouth opening is bounded ventrally by the hypopharyngeal sclerome and dorsally by a transverse sclerotization of the base of the epipharynx so that it is not easily forced to assume a round or oval shape. There are two pairs of muscles, the dilatores cibarii (*dlcb*, Figs. 6, 7, 8) which originate on the frontoclypeus and insert on the epipharynx (*Ephy*, Fig. 6). Their function is to enlarge the preoral cavity by lifting the epipharyngeal surface.

Stomodaeum

The stomodaeum (*Stom*, Fig. 1) extends from the mouth, between the circumesophageal connectives to its junction with the mesenteron in the mesothorax. It is divided into a pharynx and an esophagus. A definitive proventriculus is absent.

Pharynx

The pharynx of *C. aeripennis destructor* forms an elaborate pumping apparatus (12). The lumen (*Lum*, Figs. 2, 3, 4) is crescentic in cross section, and the intima (*In*) is thick, particularly on the ventral side, and bears two dorsally projecting apophyses (*phya*, Figs. 4 to 8). The epithelium (*Epth*, Figs. 2 to 5) rests on a basement membrane (*BM*). The pharynx is dilated

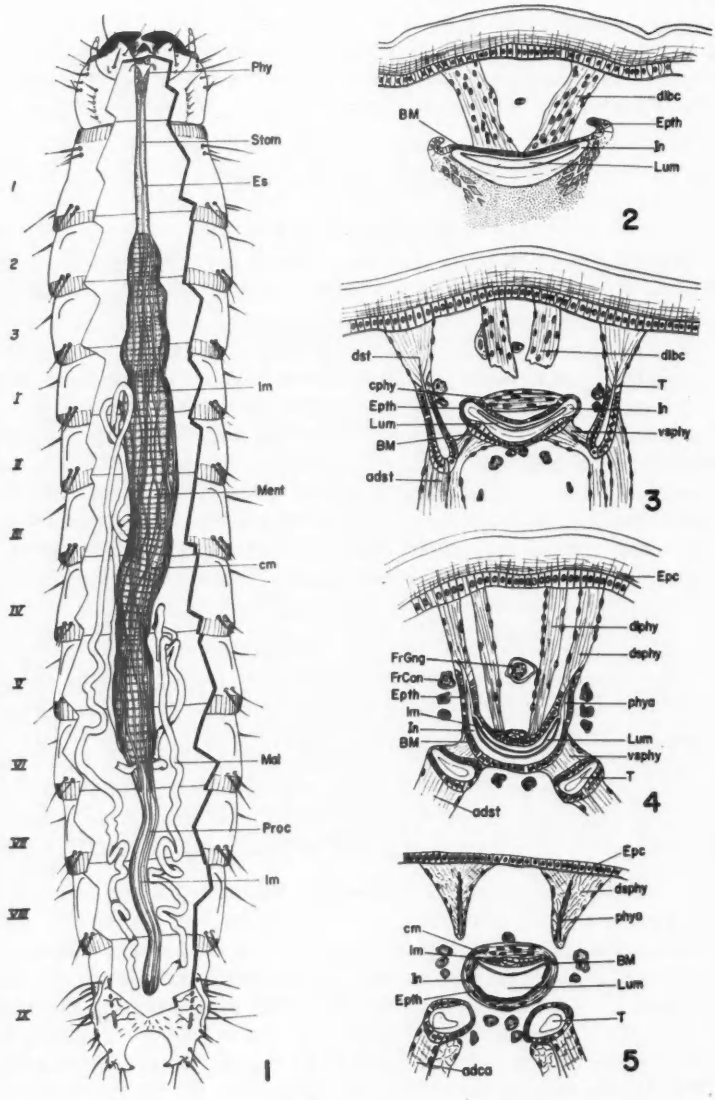


FIG. 1. Whole larva, dorsal, cut away to show digestive tract. FIG. 2. Pharynx, cross section at mouth. FIG. 3. Pharynx, cross section at second pharyngeal compressor muscle. FIG. 4. Pharynx, cross section at caudal end of frontal ganglion. FIG. 5. Pharynx, cross section behind fascicle of dilatores pharyngeales.

by the dilatores buccales (*dlbc*, Figs. 2, 3, 6, 7, 8) and the dilatores pharyngeales (*dlphy*, Figs. 4, 6, 7, 8). There are three pairs of dilatores buccales (Fig. 6), which originate on that part of the frontoclypeus which is the definitive clypeus and insert anterior to the frontal ganglion. There are six pairs of dilatores pharyngeales (*dlphy*) which originate on that part of the frontoclypeus which is the definitive frons and insert posterior to the frontal ganglion. The dilatores pharyngeales described here are, by Snodgrass' (32) definition, the dilatores pharyngis frontales. The dilatores pharyngis postfrontales, which originate on the postfrontal region of the cranium, and the dilatores postpharyngeales, which insert on the stomodaeum behind the brain (32) are absent.

The lumen is constricted by the contraction of the pharyngeal compressors (*cphy*, Figs. 3, 6), which draw the sides of the pharynx together and force the dorsal wall downward. Three of these compressors alternate with the three fascicles of the dilatores buccales. Caudad to the first fascicle of pharyngeal dilators the pharynx becomes circular in cross section and the compressors are replaced by circular muscle (*cm*, Figs. 5, 6). Five fascicles of circular muscle alternate with the fascicles of the dilatores pharyngeales.

There is one longitudinal muscle (*lm*, Figs. 4, 5) on the pharynx. It is situated on the dorsal side of the pharynx and has its anterior and posterior attachments below the frontal ganglion and between the insertions of the third pair of dilatores pharyngeales respectively. Its function is obscure, but is probably homologous with the musculus longitudinalis pharyngis anterioris of *Dytiscus marginalis* L. as described by Korschelt (25) and the dorsal longitudinal muscle of the anterior pharynx of *Periplaneta americana* (L.) as described by Eidmann (10).

The pharynx is held in place by ventral suspensors (*vsphy*, Figs. 3, 4, 8) which originate on the tentorium (*T*) and cranial dorsal suspensors (*dsphy*, Figs. 4, 5, 7) which are inserted on the pharyngeal apophyses (*phya*, Figs. 4 to 8). Snodgrass (32) gives suspensory and dilator muscles as synonymous. The homologues of the dorsal and ventral suspensors in other insects may have a dilatory function but they have clearly lost it in this larva.

James (20) does not describe the pharynx of *A. obscurus* and merely notes that "immediately behind the oral opening the gut swells slightly to form a pharynx".

Dorsey (9) described the musculature of the pharynx of the larva of *Parallelostethus attenuatus* (Say) and of *Alaus* sp. as powerful. It is essentially the same as that of the larva of *C. aeripennis destructor*. He omitted the ventral suspensors of the pharynx and the pharyngeal constrictor muscles in his drawing, but the former are present according to his description and the latter are present by implication. The pharyngeal apophyses are termed "hypopharyngeal bars Y" by Dorsey and are joined to one another by a ventral supporting area on which the ventral suspensor muscles insert. The ventral supporting area of Dorsey is the thickened intima of the ventral side of the pharynx.

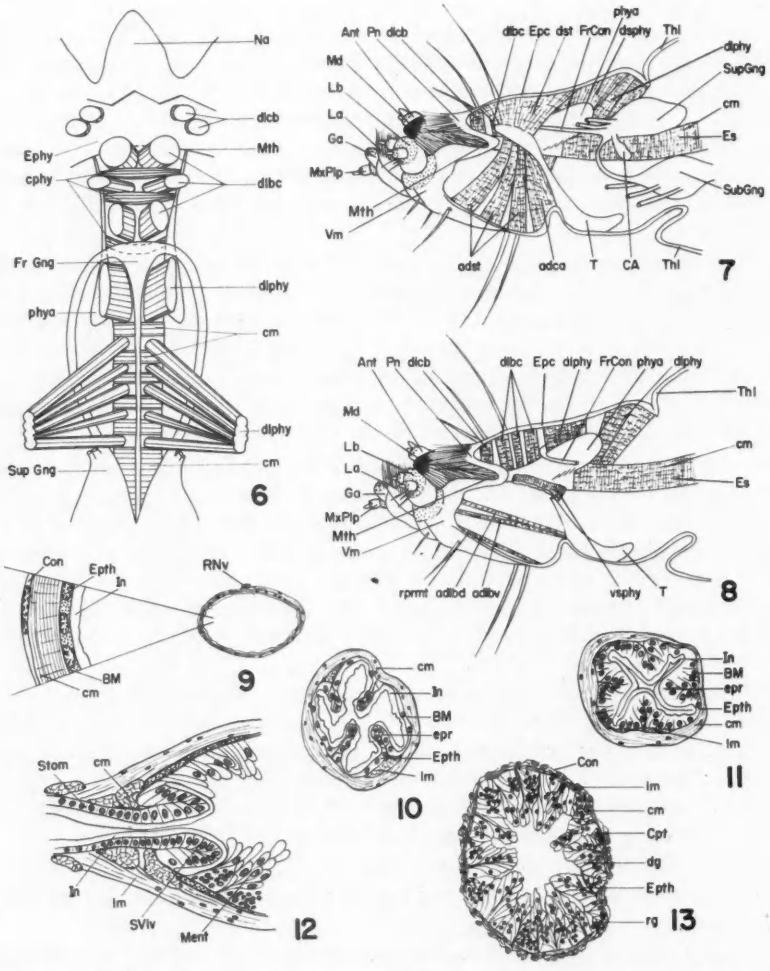


FIG. 6. Pharynx, dorsal, showing musculature. FIG. 7. Head, lateral, cut away to show pharyngeal musculature. FIG. 8. Same as Fig. 7, maxillary adductors and dorsal suspensors of tentorium and pharynx removed. FIG. 9. Esophagus, dilated, cross section, with a portion enlarged. FIG. 10. Esophagus, contracted, cross section. FIG. 11. Esophagus, proventricular region, cross section. FIG. 12. Junction of stomodaeum and mesenteron, longitudinal section, showing stomodaeal valve. FIG. 13. Mesenteron, cross section.

There are two conspicuous differences between Dorsey's description and mine. First, there are differences in the number of fascicles in each of the muscles, which is probably due to differences in observation and technique. Second, Dorsey has placed the boundary between the cibarium and the pharynx farther caudad. I have determined the location of the boundary by the insertions of the dilator muscles, in view of Snodgrass' (32) assertion that "In general the distribution of the dilator muscles serves better to identify corresponding parts of the cephalic stomodaeum than the parts themselves". The pharyngeal apophyses may very well be "hypopharyngeal bars" as Dorsey calls them. Similar apophyses which occur in *Apis mellifera* L. possibly represent the oral arms of the hypopharyngeal suspensoria of more primitive insects (32).

There are several descriptions of pharynges of insects. In Table I the pharyngeal musculature of *C. aeripennis destructor* is compared with the generalized musculature as given by Snodgrass (32) and the pharyngeal musculature of the larva of *D. marginalis* (25),* the larva of *Lampyris noctiluca* (L.) (36), the adult of *P. americana* (9), the adult of *A. mellifera* (33), and the larva of *Corydalis cornutus* (L.) (24). The compressor muscle of the anterior region of the cibarium found in *P. americana* was not found in the other insects. The dilatores pharyngis IV (externi) of *D. marginalis* are probably homologous with the dorsal suspensors of the pharynx. Dilator IV of *L. noctiluca* is probably also homologous with the dorsal suspensors of the pharynx although Vogel considers it equivalent to the dilatores pharyngis interni of *D. marginalis* as described by Rungius (29). Vogel considers the dilator VI of *L. noctiluca* equivalent to the dilatores pharyngis externi of *D. marginalis* as described by Rungius, but it originates ventrally in the head and is more likely equivalent to the ventral suspensors of the pharynx. Vogel gives the origins of dilators VI, VII, and VIII as a strongly chitinized bar which is formed from a lateral part of the maxillary stipes. It seems likely that he refers to the tentorium and all three muscles are ventral suspensors. He does not mention the tentorium elsewhere. The dilatores pharyngis externi of *D. marginalis* are probably the dorsal suspensors of the pharynx. Although Korschelt does not say what their origins are, their homologues in the adult originate on the frons. In addition to the muscles mentioned in the table, Kelsey (24) described seven other muscles which were not found in any of the other species. These are the ventral dilator of the cibarium, the ventral dilator of the anterior pharynx, the first, second, and third ventral dilators of the posterior pharynx, the ventral constrictors of the cibarium, and the constrictors of the anterior pharynx.

Esophagus

The transition from pharynx to esophagus occurs before the stomodaeum passes between the circumesophageal commissures. The esophagus (*Es*, Fig. 1) passes between the commissures and through the occipital foramen

*Korschelt drew on Rungius (29) and Burgess (5) for his interpretation of the pharyngeal musculature of *D. marginalis*.

TABLE I

HOMOLOGIES OF THE PHARYNGEAL MUSCLES

C. aeripennis destructor larva	Generalized musculature, Snodgrass (32)	*Dytiscis marginalis* larva, Korschelt (25)	*Lampyris noctiluca* larva, Vogel (36)	*Periplaneta americana* adult, Dorsey (9)	*Apis mellifera* adult, Snodgrass (33)	*Corydalis cornutus* larva, Kelsey (24)
Dilatores cibarii	Dilatores cibarii	Dilator pharyngis I and II	Dilators I and II	Dorsal dilator muscles of the cibarium (5, 6, and 7)	Retractor or levator of the epipharynx (25)	First dorsal dilator of the cibarium (35)
Dilatores buccales	Dilatores buccales	Dilator pharyngis III			Dilators of the suctorium (26–30)	Second dorsal dilator of the cibarium (36)
Dilatores pharyngeales	Dilatores pharyngis frontales			Dorsal dilator of the anterior region of the pharynx (9)	Precerebral dilators of the pharynx (34 and 35)	First and second dilators of the anterior pharynx (37 and 38); second lateral dilator of the anterior pharynx (44)
Absent	Dilatores pharyngis postfrontales	Dilator pharyngis IV (interni)	Dilators III and V	Dorsal and lateral dilator muscles of the pharynx (11 and 31)		
Absent	Dilatores postpharyngeales	Dilator pharyngis V	Not mentioned	Not mentioned	Postcerebral muscle of the pharynx (36)	First, second, and third dorsal dilators of the posterior pharynx (39, 40, and 41)
Pharyngeal compressors	Not mentioned	Musculus pharyngis transversalis	*Transversaler Kompressor*	Compressor muscle group of the cibarium (12)	Compressors of the suctorium (31)	Dorsal constrictors of the cibarium (50)
Dorsal suspensors of the pharynx	Retractores angulorum oris	Dilator pharyngis IV (externi)	Dilator IV	Muscle of hypopharyngeal bar Y (10)	Protractor and retractor of the oral plate (32 and 33)	Retractor of the mouth angle (42)
Ventral suspensors of the pharynx	Lateral and ventral dilators (not constant)	Musculi tentorio-pharyngealis	Dilators VI, VII, and VIII	Ventral dilator muscle of the pharynx (30)	Posterior and parietal contractors of the oral plate (37 and 38)	First lateral dilator of the pharynx (43)

into the thorax, where it joins the mesenteron in the mesothorax. It consists of a thin-walled tube (Fig. 9) with a delicate intima (*In*). The intima does not bear fine teeth, as described by Frenzel (14) in the larva of *T. molitor*, nor other armature. The esophageal epithelium (*Epth*) is thin, has indistinct cell walls, and rests on a basement membrane (*BM*). There is an outer layer of circular muscle (*cm*) and an inner layer of longitudinal muscle. The latter is not evident when the esophagus is dilated as in Fig. 9, but when it is contracted (Fig. 10) the epithelium is thrown up into four longitudinal ridges (*epr*) and the longitudinal muscle layer (*lm*) can be seen. The outermost layer is a thin tunic of loose connective tissue (*Con*, Fig. 9).

The epithelium of the esophagus projects into the mesenteron, forming the stomodaeal valve (*SVlv*, Fig. 12). The epithelium recurves on itself and joins the anterior end of the mesenteron; therefore the valve consists of two layers of epithelium which are covered on both sides by the stomodaeal intima. The stomodaeal muscles do not extend between the two cell layers.

According to James (20) the esophageal wall of the larva of *A. obscurus* also has four longitudinal ridges. The esophagus joins the mesenteron in the mesothorax and projects into the lumen of the mesenteron to form a stomodaeal valve. He states that the longitudinally arranged muscles are very powerful, especially in the pharynx, but he does not indicate them in his drawing. He also states that the longitudinal ridges are supplied with muscle elements from the circular muscle layer, but it is not so in *C. aeripennis destructor*.

A definitive proventriculus is absent, although the muscle layers and epithelium are somewhat thicker in this region (Fig. 11). A proventriculus was not noted in the larva of *A. obscurus* by James (20) and other coleopterous larvae such as *Altica bimarginata* Say (39), *T. molitor* (14), and *Phyllophaga gracilis* (Burm.) (13) do not have proventriculi.

Entimus nobilis (Oliv.), a curculionid, has a well-developed proventriculus according to Judd (23). Judd draws attention to several descriptions of proventriculi of curculionids and scolytids and points out that the variations in the proventricular armature have been used as a basis for classifying the Rhynchophora.

Mesenteron

The mesenteron, or ventriculus (*Ment*, Fig. 2), is not subdivided as in the larva of *A. bimarginata* (39), nor is it coiled as in *Popilius disjunctus* (Ill.) [=*Passalus cornutus* Fab.] (26). It passes from its junction with the stomodaeum in the mesothorax caudad to its junction with the proctodaeum in the sixth abdominal segment. The junction with the stomodaeum is shown in Fig. 12; the junction with the proctodaeum is shown in Fig. 16. Its diameter is four times that of the esophagus, and three times that of the proctodaeum. In fixed specimens it appears to be secondarily divided, as a result of peristaltic waves being suspended by the fixative.

The histology of the ventriculus is similar to that of the ventriculus of *A. obscurus* as described by James (20). The peritrophic membrane is absent.

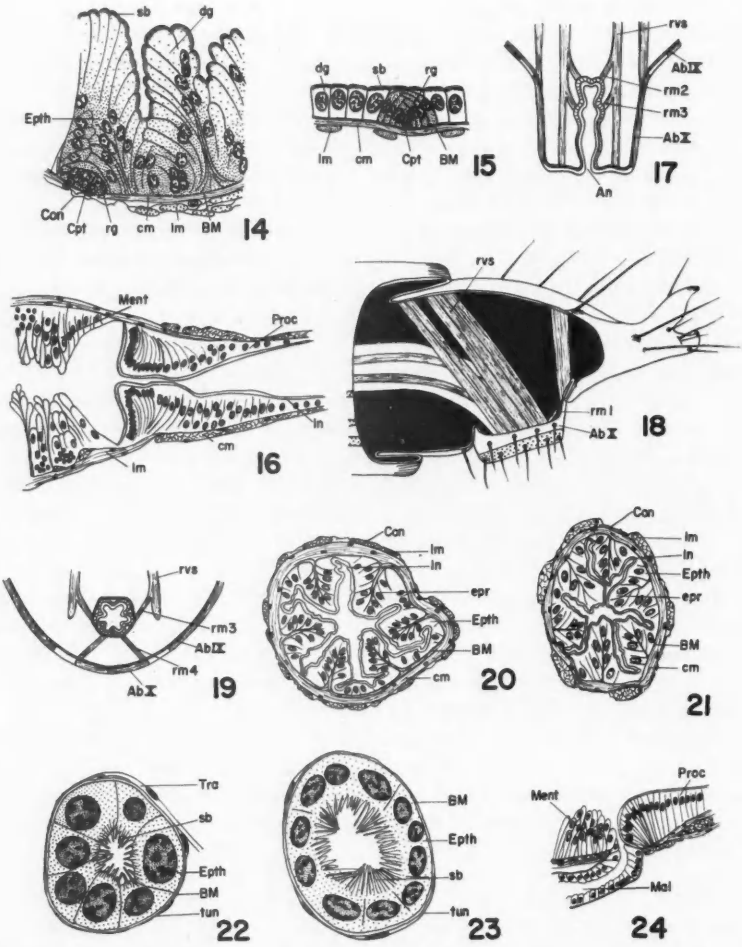


FIG. 14. Mesenteron wall, cross section, epithelium in secretory phase. FIG. 15. Mesenteron wall, cross section, epithelium in resting phase. FIG. 16. Junction of mesenteron and proctodaeum, longitudinal section. FIG. 17. Rectum and anus, cross section, diagrammatic. FIG. 18. Ninth abdominal segment, lateral, cut away to shown rectal muscles. FIG. 19. Rectum, cross section, diagrammatic. FIG. 20. Proctodaeum, anterior end, cross section. FIG. 21. Proctodaeum, cross section. FIG. 22. Malpighian tubule, distal end, cross section. FIG. 23. Malpighian tubule, proximal end, cross section. FIG. 24. Junction of Malpighian tubule and digestive tract, longitudinal section.

The epithelium is composed of two types of cells: the digestive cells (*dg*, Figs. 14, 15) and the regenerative cells (*rg*). The digestive cells do not appear to be differentiated into absorbent and secretory cells. They are columnar, with slender bases, expanded apices, and darkly staining nuclei (Fig. 14). Their basal ends stain darkly; their inner ends are highly vacuolate and bear striated borders (*sb*). The basal ends rest on a basement membrane (*BM*). The regenerative cells lie in crypts (*Cpt*) at the base of the epithelium; they have large nuclei and stain darkly with haematoxylin. They are generally regarded as the source for new cells to replace digestive cells which have been exhausted and discarded. The surface of the ventriculus is crisscrossed by strands of outer longitudinal (*lm*, Figs. 1, 14, 15) and inner circular muscle (*cm*). The outermost layer of the ventriculus is a tunic of loose connective tissue (*Con*, Fig. 14) which invests the muscles.

The epithelial cells of *A. obscurus* were described by James (20) in what he calls the secretory and quiescent phases. His drawings of digestive cells in the secretory phase resemble Figs. 13 and 14. In this phase the cells have distended apical ends and they occur in clusters which extend into the lumen of the mesenteron. At other times the digestive cells of *C. a. destructor* assume the form of a simple columnar epithelium as in Fig. 15, and according to James are in the quiescent phase. He believes that secretion in A. *obscurus* is holocrine and occurs when vesicles containing fluid bud off the apical ends of the digestive cells and are liberated in the lumen of the mesenteron.

Two types of crypts are described by Lewis (26) in the mesenteron of *P. disjunctus*. One type of crypt increases the surface area of the mesenteron and has broad epithelial cells at its base with canals opening into the lumen of the crypt. Lewis states that the secretion from these cells is merocrine, whereas the secretion from the other secretory cells of the mesenteron is holocrine. He also refers to nuclear crypts filled with darkly staining nuclei, which are glandlike evaginations of a few of the digestive crypts. He has not determined their function. Lewis does not mention regenerative cells but his drawing of a cross section of a part of the mesenteron shows cell masses that look like typical regenerative nidi.

Proctodaeum

The proctodaeum (*Proc*, Fig. 1) begins in the sixth abdominal segment, extends through the ninth, and curves ventrad into the 10th. It terminates at the slitlike anus (*An*, Fig. 17) which is on the apex of the ventrally directed 10th abdominal segment (*Ab*X). Its junction with the mesenteron is shown in Fig. 16. Fascicles of longitudinal muscle (*lm*, Fig. 1) can be observed on its surface.

Near the posterior terminus of the proctodaeum there are four pairs of suspensory (dilator) muscles. The part of the proctodaeum where these muscles are inserted, although otherwise similar to the rest of the proctodaeum, I consider to be the rectum. James (20) does not note the presence of a rectum in the larva of *A. obscurus*. The first pair of muscles (*rm*1, Fig. 18) originates on the posteroproximal margin of the 10th abdominal segment and inserts

on the dorsal side of the rectum. The second pair (*rm2*, Fig. 17) originates on the ninth abdominal tergum, and inserts on the dorsal side of the rectum. The third pair (*rm3*, Figs. 17, 19) originates on the ninth abdominal tergum and inserts on the sides of the rectum. The latter two pairs of muscles anastomose with the retractor muscles of the 10th abdominal segment (*rvs*, Figs. 17, 18, 19). The fourth pair (*rm4*, Fig. 19) originates on the antero-proximal margin of the 10th abdominal segment, and inserts on the ventral side of the rectum.

The epithelium of the proctodaeum is thrown up into six longitudinal ridges (*epr*, Figs. 20, 21) as in the larva of *A. obscurus*, as described by James (20), but the number of ridges does not vary. The epithelial cells (*Epth*) have large, prominent nuclei. They secrete an intima (*In*) at their inner ends and rest on a basement membrane (*BM*) at their basal ends. The intima does not bear armature, although some Coleoptera, for example, adults of *Popillia japonica* Newm. (35), have an armed proctodaeal intima.

The proctodaeum (Figs. 20, 21) has a well-developed inner circular muscle layer (*cm*), and an outer longitudinal muscle layer (*lm*). The longitudinal muscle layer consists of scattered muscle fibers (*lm*, Fig. 20) on the anterior tenth of the proctodaeum, and six fascicles (*lm*, Fig. 21), which alternate with the epithelial ridges, throughout the remainder of the proctodaeum. The outermost layer of the proctodaeum is a tunic of loose connective tissue (*Con*, Figs. 20, 21).

Malpighian Tubules

As in most pentamerous Coleoptera there are four Malpighian tubules (*Mal*, Fig. 1), which join the digestive tract at the junction of the mesenteron and proctodaeum. The junction is shown in Fig. 24. The left ventral and the right dorsal tubules are shown in Fig. 1; the other two are omitted for clarity. They are a little longer than the larva, but are looped and coiled, and confined to the abdomen. The ventral Malpighian tubules pass cephalad to the first abdominal segment and then caudad to the ninth, where they terminate. The dorsal tubules pass cephalad to the fourth abdominal segment, and then caudad to the ninth. All four tubules end freely in the body cavity as James (20) found in the larva of *A. obscurus*.

In many Coleoptera the distal ends of the tubules do not end freely, but penetrate beneath the outer tissues of the walls of the rectum (32). An unusual arrangement occurs in the larva of *A. bimarginata* (38). The Malpighian tubules are of two series. The first series of four vessels arises from a small ovoid "bladder" on the proctodaeum, and the second series of two vessels arises anterior to the first at the point where the mesenteron and proctodaeum join. Vessels of the second series are much shorter than those of the first. The apical ends of three tubules from each side unite to form two common trunks which become bound to the wall of the colon by connective tissue. Each common trunk redivides on the wall of the colon into three vessels, which eventually come to lie outside the circular muscles and alternate with the six fascicles of longitudinal muscle.

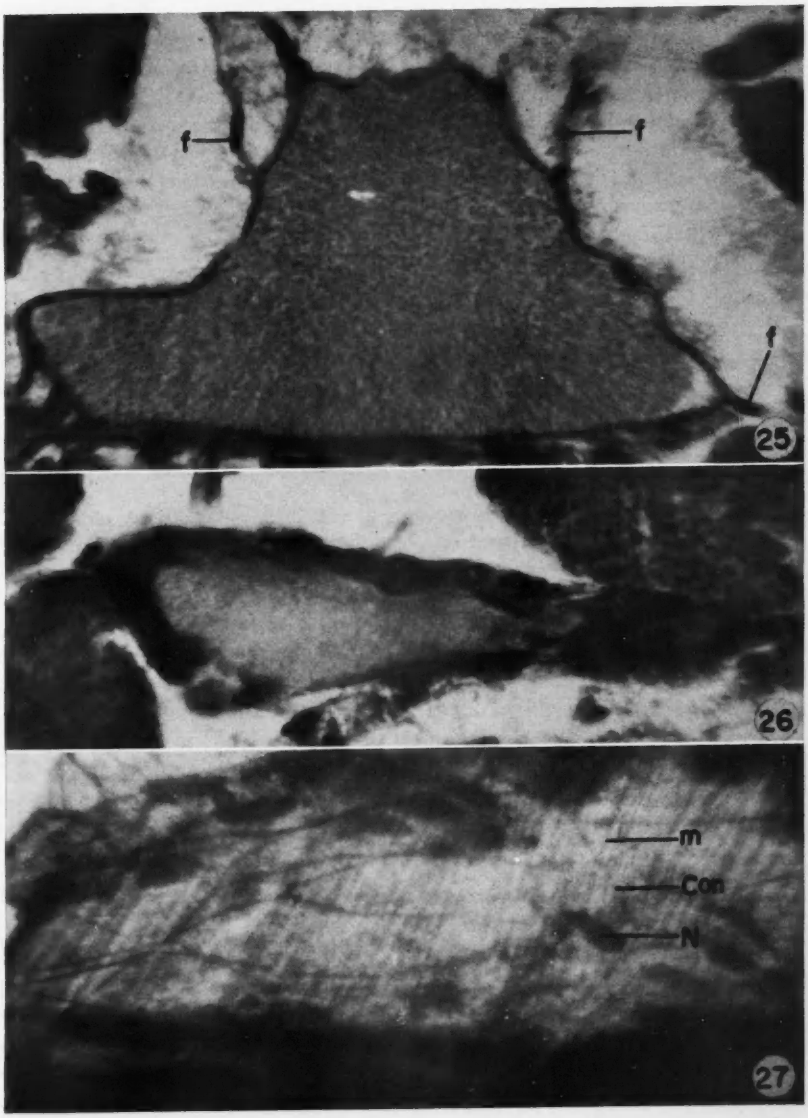


FIG. 25. Heart, dilated, cross section. FIG. 26. Heart, contracted, cross section. FIG. 27. Wall of heart.

The histology of a Malpighian tubule differs little throughout its length. The distal end has larger, more definite epithelial cells (Fig. 22) than the proximal end (Fig. 23). The epithelial cells (*Epth*) are cuboidal, with very large granular nuclei and striated inner borders (*sb.*) The striated border appears, in section, to consist of long protoplasmic processes. The epithelium rests on a basement membrane (*BM*). The basement membrane is surrounded by a connective tissue tunic (*tun*) which is richly supplied with tracheae (*Tra*). James (20) found in the larva of *A. obscurus* that some of the fibers of the proctodaeal muscle layer run along the Malpighian tubules, but this was not observed in *C. aeripennis destructor.*

Circulatory System

The circulatory system is simple and consists of a dorsal vessel and a dorsal diaphragm. The ventral diaphragm, which is present in some insects, is absent. No accessory pulsating organs are found, but Snodgrass (32) lists, from several authors, records of the occurrence of such organs in various insects. They may occur in the dorsal part of the thorax, the bases of the antennae, or in the legs.

Dorsal Vessel

The dorsal vessel (*DV*, Fig. 28) is unbranched. It begins in the ninth abdominal segment, passes cephalad into the head, and opens beneath the supraesophageal ganglion. It lies immediately under the epidermis of the median dorsal suture (*mds*, Fig. 29) between the dorsal longitudinal muscles (*d*, Figs. 28, 29).

The posterior portion of the dorsal vessel is the heart (*Ht*, Fig. 28) and is contractile. The anterior portion is the aorta (*Ao*) and may also be contractile, but this was not determined. Snodgrass (32) states that the heart is generally the pulsating part of the dorsal vessel, though the aorta frequently has pulsating vesicular diverticula. Wigglesworth (37) states that both the heart and aorta of insects are contractile. The aorta of *C. aeripennis destructor* probably pulsates actively, because it has muscular walls similar to those of the heart.

Heart

The heart (*Ht*, Fig. 28) extends from the ninth to the first abdominal segment. Its chambers are indistinct, but they are easily seen when it pulsates. There are eight chambers, each one distinctly defined by the insertions of the alary muscles. Each chamber has an ostium on each side near the posterior end. The ostia do not function as valves between chambers. Valves are occasionally developed from folds of the inner wall of the heart, independent of the ostia (37), but no such valves are present in this species.

The heart consists of a muscular tube. The muscle fibers (*m*, Fig. 27) wind in a sinistral spiral about the vessel in a manner similar to those of the larva of *Anopheles quadrimaculatus* Say (22). The pitch of the spiral is about 40° to 45° in *A. quadrimaculatus* (measured from Jones's (22) photomicrographs) and about 20° in *C. aeripennis destructor.* A few muscle fibers

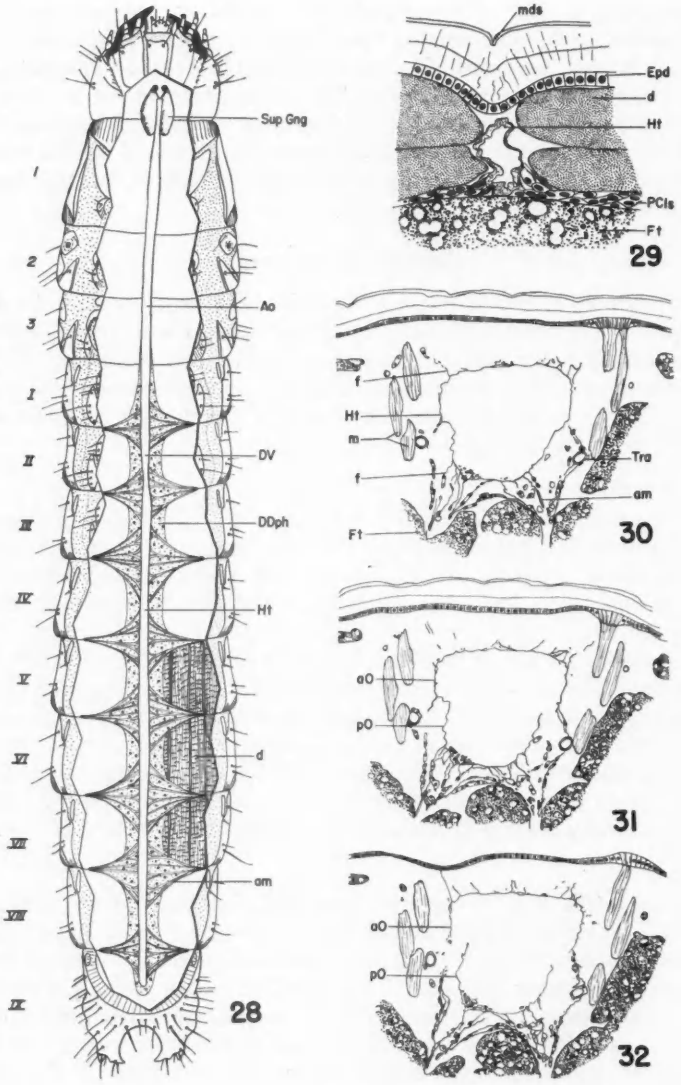


FIG. 28. Whole larva, ventral, cut away to show dorsal vessel. FIG. 29. Heart, contracted, cross section. FIGS. 30 to 32. Heart, serial cross sections to show ostia.

are also visible in Fig. 26. The heart is not lined by endothelium and the sarcoplasm of the heart muscle forms the innermost layer. Outside the heart there is a delicate network of connective tissue fibers (*Con*, Fig. 27). The nuclei (*N*) also probably belong to the connective tissue layer. The heart is joined to the tergum and to the dorsal diaphragm by fine radiating fibers (*f*, Figs. 25, 30), which appear to be derived from the connective tissue sheath of the heart walls. Figs. 25 and 26 are cross sections showing the heart dilated and contracted respectively.

There are eight pairs of ostia, one pair near the caudal end of each heart chamber. They are small slits in the sides of the heart. Figs. 30 to 38 are drawings of cross sections in serial sequence progressing caudad through the heart at the eighth pair of ostia. Fig. 30 shows a section immediately anterior to the ostia. The gradual appearance of the posterior margins (*pO*) is shown in Figs. 31 to 36; the gradual disappearance of the anterior margins (*aO*) is shown in Figs. 34 to 38. The anterior margins appear to be somewhat heavier than the posterior margins, indicating that they are more or less rigid, whereas the posterior margins are flexible. The flexibility of the posterior margins permits the ostia to open and close during diastole and systole respectively.

Figure 39 is a diagrammatic reconstruction from serial sections of the heart at the eighth pair of ostia. Connective tissue fibers (*fa*) anchor the heart to the terga, and other fibers (*fb*) secure flaps of the anterior margins of the ostia to the dorsal diaphragm. These fibers undoubtedly assist in holding the anterior margin rigid during systole.

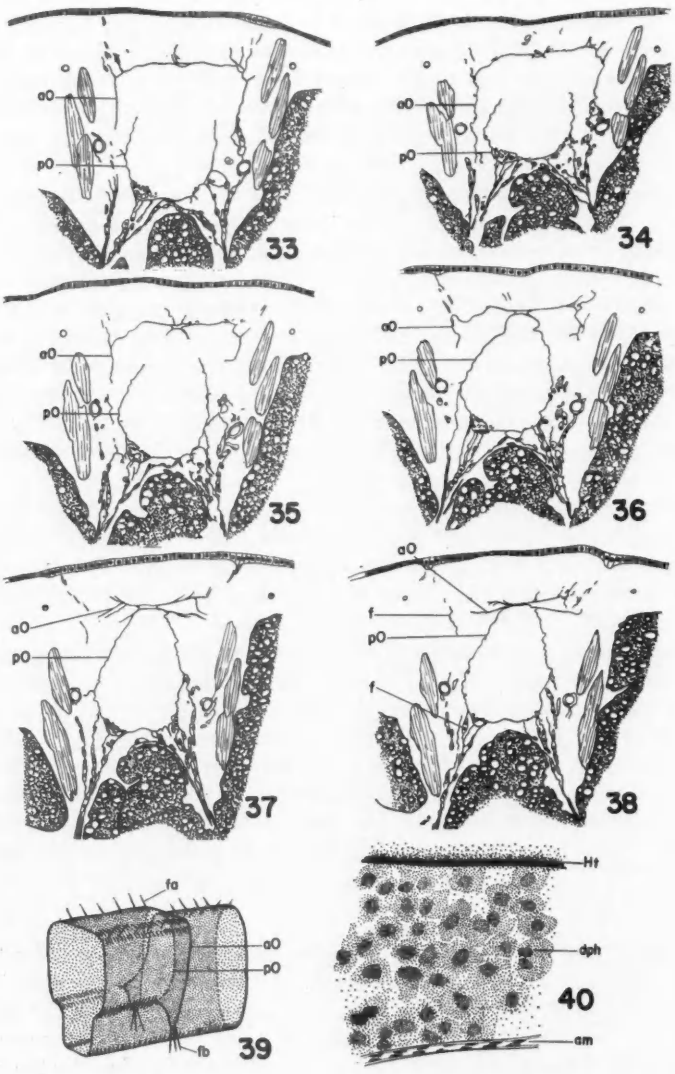
Typical cardiac ostia usually have the form of vertical or oblique slits in the sides of the heart (32). Their margins are prolonged inward to form valves, which prevent the reflux of blood into the body cavity when the heart contracts (37). The ostia of *Dytiscus marginalis* are of this type. When the heart is dilated the ostia are simple slits, but when the heart is contracted their margins fold inwards forming valves (25). The ostia of the larva of *C. aeripennis destructor* are more complex and close in a different manner. Their margins do not fold inward during any phase of the pulsating cycle.

Aorta

The aorta (*Ao*, Fig. 28) differs from the heart in the absence of ostia and alary muscles, and it is not chambered. It begins in the first abdominal segment at its junction with the heart and opens below the supraesophageal ganglion. The diameters of the heart and aorta do not differ perceptibly, but the point of junction with the heart can easily be determined by the absence of alary muscles on the aorta. The aorta has the same histological structure as the heart.

Dorsal Diaphragm

The dorsal diaphragm (*DDph*, Fig. 28) is composed of flat layers of connective tissue and striated muscle fibers (alary muscles). It is confined to



FIGS. 33 to 38. Heart, serial cross sections to show ostia. FIG. 39. Ostia, diagrammatic reconstruction from serial sections. FIG. 40. Cells of dorsal diaphragm.

the abdomen and occurs in the same segments as does the heart. The connective tissue cells of the dorsal diaphragm (*dph*) are shown in Fig. 40, which is a dorsal view of a portion of the dorsal diaphragm. Cut perpendicular to the longitudinal axis of the heart, these cells (*dph*, Fig. 41) appear very flat. The dorsal diaphragm separates the dorsal sinus or pericardial sinus (*DS*, Fig. 43) from the perivisceral sinus. It functions as an important adjunct to the dorsal vessel in the circulation of the blood (32). When the alary muscles contract, the dorsal diaphragm enlarges the dorsal sinus, displaces blood towards the heart, and aids in the dilation of the heart (38). Wigglesworth also points out that it controls, in part, the direction of the blood stream outside the heart.

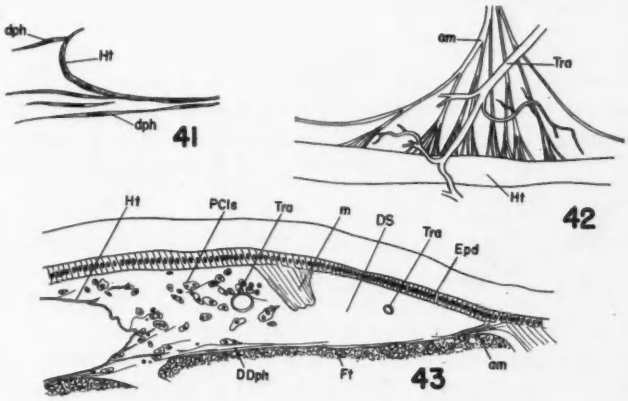
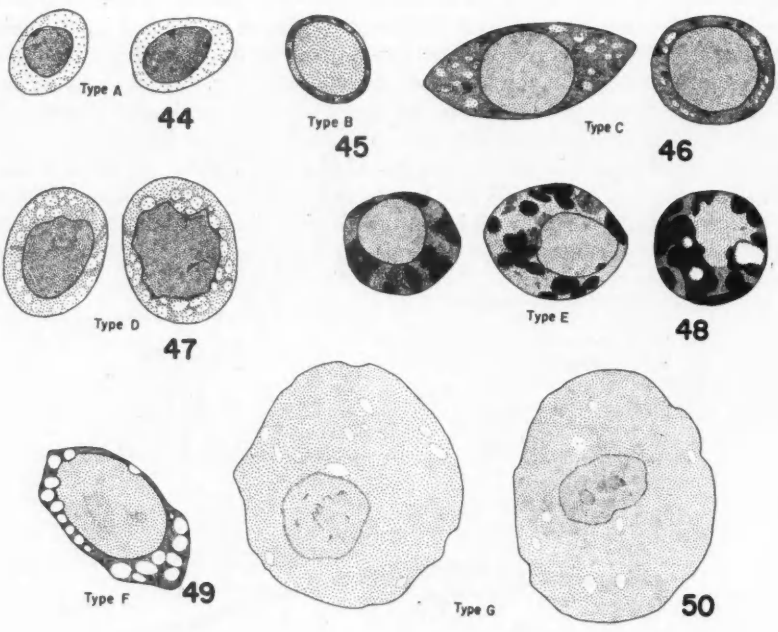


FIG. 41. Portion of heart wall, cross section. FIG. 42. Alary muscles. FIG. 43. Dorsal sinus and dorsal diaphragm, cross section.

The alary muscles (*am*, Figs. 28, 42) are fan-shaped groups of fibers, which take their origins on the tergal conjunctivae and are inserted on the ventral wall of the heart. There are eight pairs, one for each chamber of the heart. The dorsal diaphragm is imperforate, but large gaps occur between the origins of the alary muscles, leaving openings through which the dorsal sinus and the perivisceral sinus communicate. Fig. 43 is a cross section of half the dorsal sinus (*DS*) and half the dorsal diaphragm (*DDph*), and shows the origin of an alary muscle (*am*). The large cells in the dorsal sinus are pericardial cells (*PCls*).

Blood

Paillot (27), in a comprehensive study of the blood cells of insects, examined the blood cells of elaterid larvae but he did not say what species he examined. He found that the blood cells exist in two main forms but this is certainly not the case in the larva of *C. aeripennis destructor*. It may be, as Yeager (40) suggests, that Paillot's classification is too simplified and of too general a



FIGS. 44 to 50. Blood cell types.

nature. Jones (21) described the blood cells of *Tenebrio molitor* in all stages, using, with slight modifications, the classification that Yeager (40) developed in his study of the blood cells of *Prodenia eridania* (Cram.). Jones's classification of the blood cells of *T. molitor* is very detailed; in the blood of the larva he distinguishes seven types, which he splits into 22 subtypes. The blood cells of *C. aeripennis destructor* were not studied in sufficient detail to attempt to fit them to Jones's classification. Arnold (2) bases his classification of the blood cells of *Anagasta kühniella* (Zell.) on Yeager's classification, but it differs considerably from the original. He omits Yeager's cell "types" ("subtypes" of Jones) because they represent transitional forms, and omits certain of his cell "classes" ("types" of Jones) for the same reason.

"A comprehensive study of insect haemocytes sufficiently detailed to be applied directly to the cells of different species has not yet been made" (2). The blood cells, or hemocytes, described below are therefore not named, but are distinguished as types A to G. They are compared with Arnold's cell classes.

The blood of *C. aeripennis destructor* consists of a matrix of colorless fluid containing cellular elements in a variety of forms. Seven types of blood cells can be distinguished.

Type A

About 18 per cent of the blood cells look like those in Fig. 44, and are called type A here. Cells of this type have small nuclei and homogeneous hyaline cytoplasm. They are round to oval and 6 to 9 microns in diameter. These cells seem quite different from the other types and may be the oenocytoids.

Of the types described in the many classifications that have been proposed, oenocytoids and proleucocytes seem to be the only blood cell types that are well established. Although the type A cells do not fit Arnold's description of the oenocytoids of *A. kühniella* perfectly, because of their small nuclei, homogeneous cytoplasm, and distinctive appearance, they probably belong to this group.

Type B

A cell of type B (Fig. 45) has a large nucleus and scant, deeply staining cytoplasm. The cytoplasm is not granular but contains dense patches. These cells are round and about 9 microns in diameter. They are undoubtedly the prohemocytes of Arnold's classification. Cells of this type are called proleucocytes by Wigglesworth (37).

Type C

Type C cells (Fig. 46), together with the type B cells, with which they intergrade, comprise about 54 per cent of the blood cells. They are round to fusiform and 6 to 17 microns in diameter, although most are 11 to 12 microns in diameter. A cell of this type has a large nucleus and a variable amount of basophilic cytoplasm which frequently contains small vacuoles and small granules. They are probably a form of plasmatocyte according to Arnold's classification, or a type of macronucleocyte according to Wigglesworth's classification.

Type D

Type D cells (Fig. 47) comprise about 18 per cent of the blood cells. Most are round and 9 to 10 microns in diameter. Their nuclei are frequently of irregular shape, but otherwise resemble those of type C cells. They are probably a form of plasmatocyte.

Type E

Type E cells (Fig. 48) comprise about 5 per cent of the blood cells. They have the same shape and size range as type C cells, but most of them are 12μ in diameter. They differ from type C cells by the opaque and nearly opaque granules contained in the cytoplasm. They are probably the granular leucocytes of Wigglesworth's classification. According to Wigglesworth granular leucocytes are present in Coleoptera and are perhaps just phagocytes (a group that includes macronucleocytes and micronucleocytes) loaded with granules of different sorts. Sometimes the cytoplasm of type E cells also contains a large vacuole; sometimes a few small ones as well. In Arnold's classification they are probably a type of plasmatocyte.

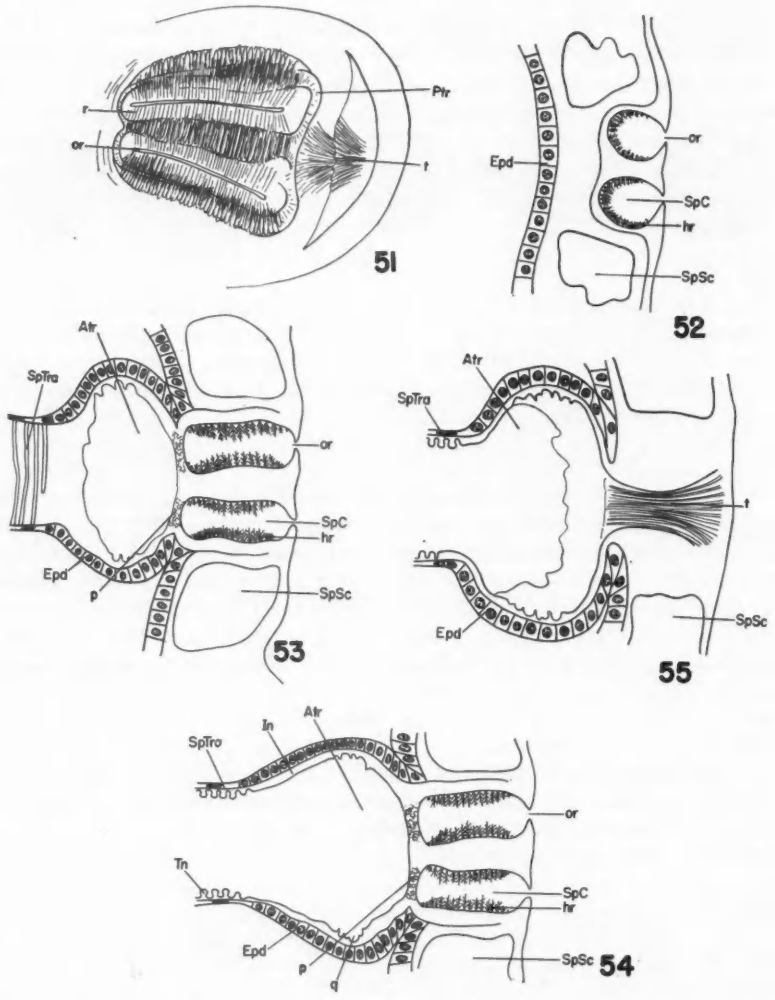


FIG. 51. Right abdominal spiracle. FIGS. 52 to 55. Right abdominal spiracle, successive cross sections progressing cephalad.

Type F

Type F cells (Fig. 49) comprise about 2 per cent of the blood cells. They are similar to type C cells except that they are larger and their cytoplasm contains many vacuoles.

Arnold describes cells, which he calls spheroidocytes, in the larvae of *A. kühniella* and Wigglesworth describes cells which he calls spherule cells adding that there is little reason to doubt that they are derived from phagocytes. Spheroidocytes and spherule cells are probably the same type, and type F cells probably belong to this group.

Type G

Type G cells (Fig. 50) are very distinctive, large, irregular cells, 14 to 24 microns in diameter. They comprise about 3 per cent of the blood cells. They have abundant, homogeneous, basophilic cytoplasm with a few small vacuoles. Their nuclei are irregular in shape, rather indistinct, and stain lightly. They may be degenerating cells, but their actual identity according to existing classifications has not been determined.

Respiratory System

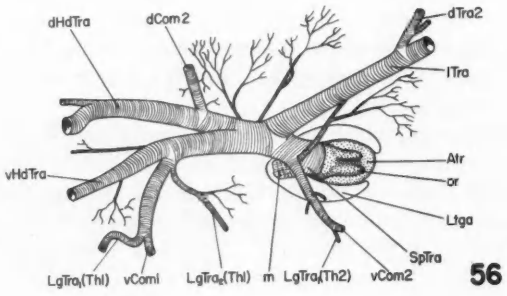
Spiracles

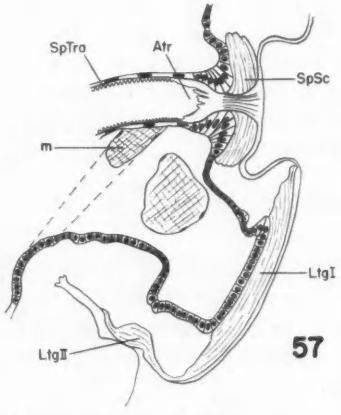
There are nine pairs of spiracles. The only thoracic pair, the mesothoracic spiracles, is located on the anterior laterotergites of the mesothorax. The abdominal spiracles are smaller than those of the thorax and are located on small laterotergites, termed spiracular sclerites by Glen (15), on each side of the first eight abdominal segments.

Except for size, the thoracic and abdominal spiracles are similar. They are widest at the anterior end and are biforate. There are no teeth on the sides of the orifices (*or*, Fig. 51) as was found by Roberts (28) in the spiracles of *Agriotes obscurus* larvae. The peritreme bears ridges (*r*) in both *C. aeripennis destructor* and *A. obscurus*, but in the latter the ridges continue beyond the intervals, forming teeth. Anterior to the orifices there is a dark sclerotic thickening (*t*, Figs. 51, 55) similar to that of the spiracle of *Alaus oculatus*, as described by Snodgrass (32).

Figures 52 to 55 are drawings of a series of sections of a right abdominal spiracle, progressing cephalad. The orifices (*or*, Figs. 52, 53, 54) of the spiracle lead to the spiracular chambers (*SpC*), which are elongate cavities in the cuticle lined with dense brushes of branched hairs (*hr*). The spiracular chambers are separated from the atrium (*Atr*, Figs. 53, 54, 55) by a trabeculated septum (*p*, Figs. 53, 54), and air entering the atrium evidently must diffuse through this septum. The atrium has a large globose lumen. Its intima is thin in one place (*q*, Fig. 54), possibly permitting collapse of the atrium.

In the thorax, a short muscle (*m*, Fig. 56) originates on the anterior laterotergite (*Ltga*) and is inserted on the anterior side of the spiracular trachea





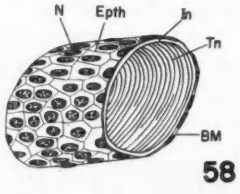


FIG. 56. Right thoracic spiracle from inside larva. FIG. 57. Right abdominal spiracle, cross section through closed primary orifice. FIG. 58. Trachea.

(*SpTra*) near its junction with the atrium. A similar muscle (*m*, Fig. 57) is associated with each of the abdominal spiracles. Its origin is on the pleural membrane between the second laterotergite (*Ltg* II) and the sternum and its insertion is anteroventral on the spiracular trachea. The function of these muscles is unknown.

Snodgrass (32) describes a larval spiracle of *A. oculatus* which is structurally similar to a larval spiracle of *C. aeripennis destructor*. According to Snodgrass, the anterior dark thickening marks the site of the closed atrial orifice, and the functional openings are secondary. Each secondary opening leads to a shallow cuticular chamber (called "spiracular chamber" above), whose inner wall is adnate with the outer wall of the closed atrium. Snodgrass adds that it has often been claimed these chambers are closed cavities and that observed openings are artifacts, but he found through manipulation that in *A. oculatus* the openings do exist. The openings also exist in the larval spiracles of *A. obscurus* (28) and *C. aeripennis destructor*. At each ecdysis the primary atrial orifice opens to permit the withdrawal of tracheal exuviae, and the entire spiracular structure is renewed (32).

Tracheae

The tracheae conform histologically to the type most common in insects. There are no air sacs. The intima (*In*, Fig. 58) is thrown into spiralling ridges, the taenidia (*Tn*), which make a few turns around the tracheal wall and then terminate. The taenidia do not bear hairs nor other processes, and are not jointed. The epithelium (*Epth*) consists of squamous polygonal cells with bulging nuclei (*N*) and rests on a basement membrane (*BM*). The layers of the tracheal wall are continuous with the corresponding layers of the body wall.

The tracheal system follows the general plan of body tracheation as described by Snodgrass (32). A short spiracular trachea (*SpTra*, Figs. 59, 60) extends inward from each spiracle and gives off three main branches. The first is a dorsal branch which tracheates the muscles of the dorsal body wall and the dorsal vessel, and joins its counterpart of the opposite side to form a dorsal commissure (*dCom*, Fig. 60); the second is a ventral branch which tracheates the muscles of the ventral body wall and the ventral nerve cord, and joins its counterpart of the opposite side to form a ventral commissure (*vCom*, Fig. 59); the third, the visceral trachea (*VsTra*, Fig. 60), tracheates the digestive tract, fat body, and in the appropriate segments, the gonads and genital ducts. The spiracular tracheae of successive segments are joined by a pair of longitudinal trunks (*lTra*, Fig. 59, 60). Dorsal longitudinal trunks, ventral longitudinal trunks, and visceral longitudinal trunks, which are sometimes present in insects (32), are absent in *C. aeripennis destructor*.

The branching of the finer tracheae in *C. aeripennis destructor* varies from individual to individual, from segment to segment, and on opposite sides of the same segment. The tracheae discussed here are always present, although the position of their origins and the manner in which they branch vary. This variation is well illustrated by the origins of the visceral tracheae (*VsTra*) in the second to seventh abdominal segments in Figs. 59 and 60. The distribution of the tracheae is not normally as symmetrical as shown.

Tracheation of the Head

Two large tracheae enter the head from each mesothoracic spiracle. The first, the dorsal head trachea (*dHdTra*, Fig. 60), passes lateral to the supraesophageal ganglion and branches profusely in the dorsal part of the head. The dorsal head tracheae of each side are joined behind the supraesophageal ganglion by the dorsal commissure of the prothorax (*dCom*1), which tracheates the supraesophageal ganglion. The second, the ventral head trachea (*vHdTra*, Fig. 59), passes ventral to the supraesophageal ganglion and esophagus and lateral to the tentorium, and branches profusely in the ventral part of the head. The ventral head tracheae are joined by a commissure (*vComHd*) where the head joins the prothorax. The arrangement of the finer branches of the head tracheae is not constant, but the mandibles, maxillae, and labium are always tracheated by the ventral head tracheae.

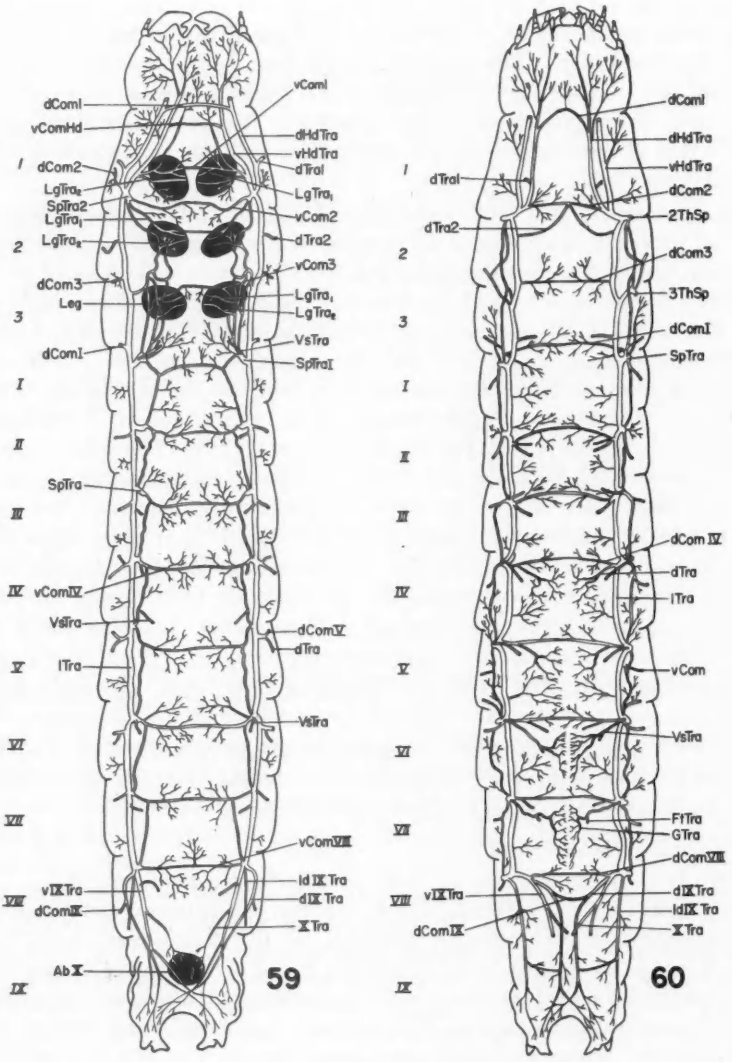


FIG. 59. Tracheae, dorsal dissection. FIG. 60. Tracheae, ventral dissection.

Korschelt (25) found that two pairs of tracheae enter the head of the larva of *D. marginalis*, and he described their finer branches in detail. To attempt to give such a detailed description of the branches of the head tracheae in the larva of *C. aeripennis destructor* would be of little value because, as Snodgrass (32) points out, the tracheal system of insects readily develops new branches to such an extent that attempts at comparative studies have been fruitless.

Tracheation of the Thorax

The prothoracic spiracle is absent and the head and prothorax are tracheated from the mesothoracic spiracle. In this larva the prothoracic tracheae arise on the head tracheae, but in the caterpillar (as described by Snodgrass (32)) the prothoracic tracheae arise close to the mesothoracic spiracle. This is because the mesothoracic spiracles are located in the mesothorax in the wireworm rather than in the prothorax as in the caterpillar. The dorsal commissure of the prothorax (*dCom*1, Fig. 60) joins the dorsal head tracheae (*dHdTra*) just before they enter the head. The ventral commissure (*vCom*1, Fig. 59) joins the ventral head tracheae (*vHdTra*) in the thorax. Each prothoracic leg has two tracheae: the first ($LgTra_1$) is a branch of the ventral commissure (*vCom*1); the second ($LgTra_2$) originates on the ventral head trachea (*vHdTra*).

In the mesothorax, the lateral tracheal trunks (*lTra*, Fig. 59) are joined by a ventral commissure (*vCom*2). The dorsal commissure of the mesothorax (*dCom*2, Fig. 60) joins the dorsal head tracheae (*dHdTra*) near their bases. Each mesothoracic leg is served by two tracheae. The first ($LgTra_1$, Fig. 59) arises on the spiracular trachea of the thoracic spiracle, and joins the first leg trachea of the other side by a fine commissure. The second ($LgTra_2$) is formed by the union of two tracheae: one arises on the spiracular trachea of the thoracic spiracle; the other arises on the lateral trunk where a metathoracic spiracle would be. (In the specimen drawn in Fig. 60, a rudimentary left metathoracic spiracle (*3ThSp*) was found.) The larger tracheae arising from the thoracic spiracle are shown in detail in Fig. 56.

The metathoracic spiracles are normally absent. The dorsal part of the metathorax is tracheated from the dorsal commissure (*dCom*3, Fig 60), which arises from the lateral trunks (*lTra*). The arrangement of the tracheae of the ventral side of the metathorax is not constant; one arrangement is shown in Fig. 59. The ventral part of the segment is tracheated, in part, by the ventral commissure (*vCom*3, Fig. 59). The ventral commissure is formed by the union of two tracheae from each side: one arises near the posterior origin of the second mesothoracic leg trachea; the other arises on the spiracular trachea of the first abdominal spiracle (*SpTra*I). The first metathoracic leg trachea ($LgTra_1$) arises on the ventral commissure (*vCom*3). The second metathoracic leg trachea ($LgTra_2$) is formed by the union of two tracheae: one arises near the posterior origin of the second mesothoracic leg trachea; the other arises on the spiracular trachea of the first abdominal spiracle (*SpTra*I).

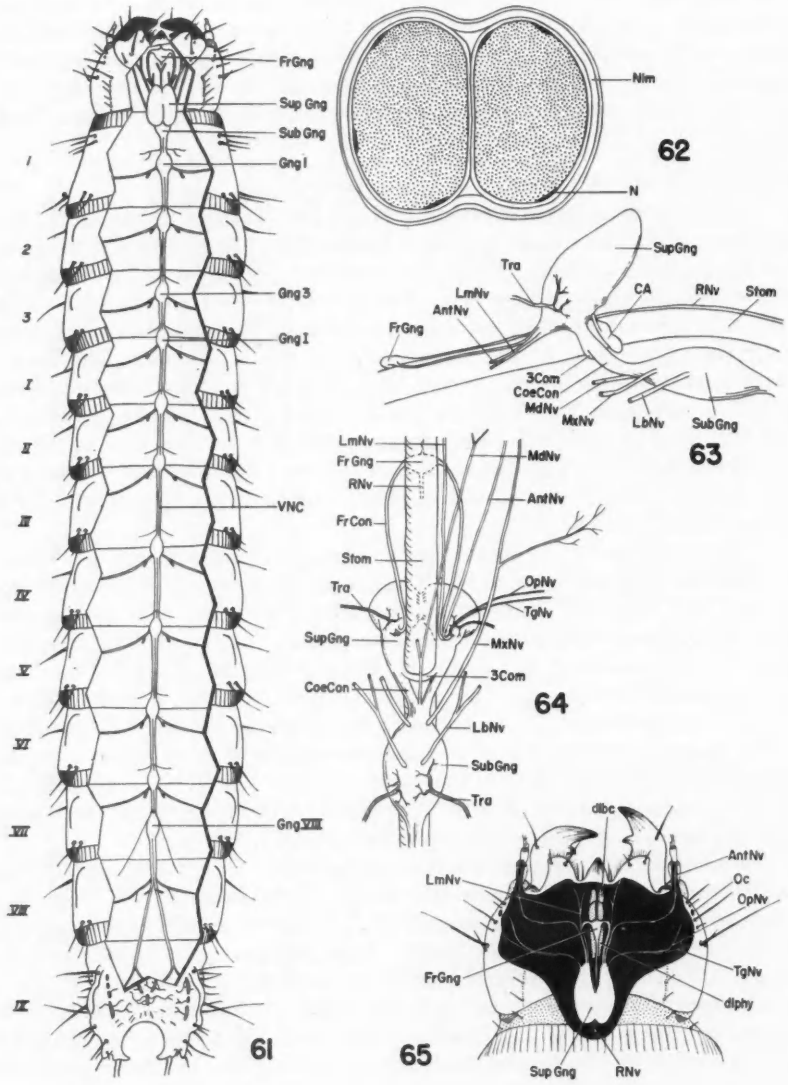


FIG. 61. Whole larva, dorsal, cut away to show ventral nerve cord. FIG. 62. Ventral nerve cord, cross section. FIG. 63. Ganglia and nerves of head, lateral. FIG. 64. Ganglia and nerves of head, ventral. FIG. 65. Nerves of supraesophageal ganglion, dorsal.

Tracheation of the Abdomen

The tracheae are arranged in a similar way in each of the first seven abdominal segments, with the exception that two mesothoracic tracheae arise from the first abdominal spiracle. Each segment has a ventral commissure (*vCom*, Fig. 59) which tracheates the ventral part of the segment and the ventral nerve cord, and a dorsal commissure (*dCom*, Fig. 60) which tracheates, in part, the dorsal part of the segment and the dorsal vessel. Each ventral commissure (*vCom*, Fig. 59) is formed by the union of two tracheae from each side: one trachea arises on the spiracular trachea; the other arises on the spiracular trachea of the following segment. Each dorsal commissure (*dCom*, Fig. 60) usually has a branch on each side (*dTra*), which tracheates, in part, the dorsal longitudinal muscles. There are paired visceral tracheae (*VsTra*) in each segment which divide into two branches, one which tracheates the fat body (*FtTra*) and one which tracheates the gut (*GTra*). The origins of the visceral tracheae vary in position and may be on the spiracular tracheae, on the lateral trunks, or on either root of the ventral commissures.

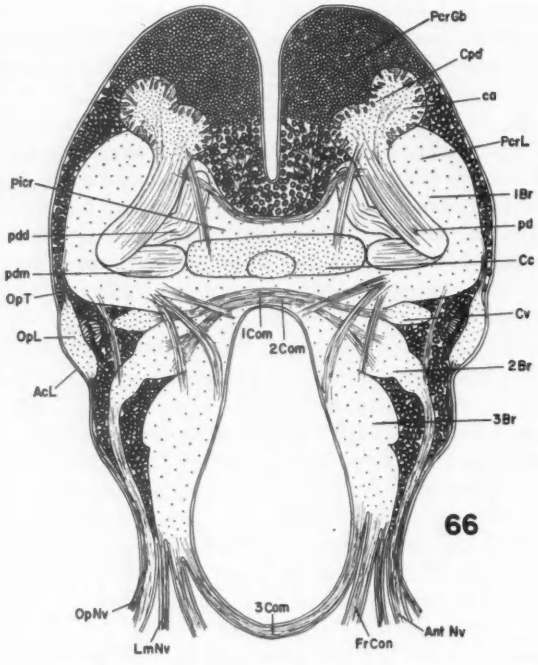
The ninth and 10th segments are tracheated from the eighth abdominal spiracle. A large paired ventral trachea (*v*IX*Tra*, Figs. 59, 60) serves the ninth and 10th segments. It bifurcates in the eighth segment and one branch serves the ventral part of the ninth segment. The other branch (X*Tra*) serves the 10th segment and rectum. The dorsal part of the ninth segment is tracheated by two paired tracheae which have a common origin on the spiracular trachea of the eighth segment: the dorsal ninth abdominal trachea (*d*IX*Tra*, Fig. 60), and the lateral dorsal ninth abdominal trachea (*ld*IX*Tra*). The former is joined to its counterpart of the opposite side by a commissure (*dCom*IX) in the eighth segment; the latter is joined to the dorsal ninth abdominal trachea by a fine branch in the specimen drawn in Fig. 60.

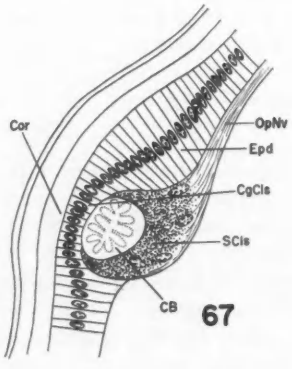
The eighth abdominal segment is tracheated by fine branches from the ninth abdominal tracheae, the ventral commissure (*vCom*VIII, Fig. 59), and the dorsal commissure (*dCom*VIII, Fig. 60).

Nervous System

The central nervous system (Fig. 61), like that of primitive insects, shows little fusion of the ganglia (*Gng*) of the ventral nerve cord. There are three thoracic and eight abdominal ganglia which, according to Wigglesworth (37) is characteristic of primitive insects such as *Machilis*. The connectives are partly fused, except between the subesophageal and third thoracic ganglia, where they are distinctly separate for most of their lengths. Where the two connectives are partly fused, the neurilemma (*Nlm*, Fig. 62) is composed of two layers: one surrounds each connective, the other surrounds the whole nerve cord. The innermost layer is a very thin layer of cells with flattened nuclei (*N*), the outer, a thick non-nucleated layer which firmly supports the nervous tissue.

Little cephalization has taken place. Using ganglion length as a unit, as did Beier (3), the first thoracic ganglion is only one unit from the subeso-





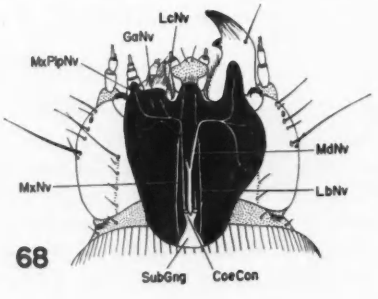


FIG. 66. Supraesophageal ganglion, longitudinal section. FIG. 67. Ocellus, cross section. FIG. 68. Nerves of subesophageal ganglion, dorsal.

phageal ganglion and one and one-half from the second thoracic. The second thoracic ganglion is two and one-third units from the third thoracic. The first seven abdominal ganglia lie partly or wholly in the segment preceding the one to which they belong. Hence the first abdominal ganglion is only one and one-third units from the third thoracic, whereas the remaining connectives are from two to three units long. The eighth abdominal ganglion is slightly larger than the other abdominal ganglia, being a composite of the ganglia of the eighth, ninth, and 10th abdominal segments. It is located in the seventh abdominal segment, only one ganglion length from the seventh abdominal ganglion.

This species may be compared with *Alaus oculatus* and *Elater sanguineus*, whose central nervous systems were described by Beier (3). *C. aeripennis destructor* has the shortest connectives between the subesophageal and the first thoracic ganglia. *A. oculatus* has the shortest connectives and *E. sanguineus* the longest between the seventh and eighth abdominal ganglia. Both *A. oculatus* and *E. sanguineus*, however, have shorter connectives than *C. aeripennis destructor* between the third thoracic and first abdominal ganglia. Cephalization that has occurred seems to be along different lines in these three species.

Supraesophageal Ganglion

The supraesophageal ganglion (*SupGng*, Fig. 61), or brain, is located mostly in the head, with a part extending into the thorax. As a result of the prognathous condition of this larva, it is tilted back so that the part that is dorsal in hypognathous insects is directed caudad. In Fig. 66 it is shown with the posterior end at the top of the page because, oriented this way, it is easily compared with other authors' drawings of brains of other insects. In Fig. 63 it has been tilted forward about 45 degrees to show the corpora allata. Its external form is bilobed (*SupGng*, Fig. 65); the surface is white, smooth, and shiny.

The supraesophageal ganglion is composed of three main regions. The first and largest is the protocerebrum (1*Br*, Fig. 66), which occupies the posterior part of the brain. The second is the deutocerebrum (2*Br*) which lies between the protocerebrum and the tritocerebrum (3*Br*), the third and anteriormost region. The protocerebrum and the deutocerebrum belong to the primitive preoral segments and hence their commissures (1*Com* and 2*Com*, respectively) pass above the esophagus and within the brain. Morphologically the tritocerebral lobes of the brain are primarily postoral in position (33); therefore the commissure (3*Com*) passes beneath the esophagus, as an externally visible nerve.

Protocerebrum

The protocerebrum is the largest and most complex region of the supraesophageal ganglion, being composed of a number of association centers surrounded by globuli cells. It includes the lateral protocerebral lobes (*PcrL*), the median pars intercerebralis (*Picr*), and the anterolateral accessory lobes (*AcL*).

The corpora pedunculata (*Cpd*) are the most prominent of the association centers. Each has a long bifid pedunculus (*pd*), a calyx (*ca*), and a large cluster of protocerebral globuli cells (*PcrGb*) posterior to the calyx. The pedunculus of each corpus pedunculatum has a median root (*pdm*) and a dorsal root (*pdd*). The median root runs mesad towards the corpus centrale (*Cc*); the dorsal root runs mesad, dorsad, and caudad.

The corpus centrale (*Cc*) is a prominent association center, located in the center of the protocerebrum.

The accessory lobes (*AcL*) are slight prominences on the surface of the supraesophageal ganglion, under which the optic lobes (*OpL*) and the corpora ventralia (*Cv*) lie. The corpora ventralia are small anterior association centers, joined by the protocerebral commissure (*1Com*). The optic lobes (*OpL*) are small and are joined to the protocerebral lobes and to one another by the optic tract (*OpT*). A group of darkly staining cells, which appear to be columnar, lie beneath the optic lobes. These cells are probably precursors of some part of the adult compound eyes. The optic nerves (*OpNv*) originate in the optic lobes, and run under the neurilemma to emerge as definitive nerves at the anterior end of the brain.

Most of the features of the insect brain, as described by Snodgrass (32), were found in the supraesophageal ganglion of the larva of *C. aeripennis destructor*. The pons cerebralis was not seen and the three association centers of the optic lobes, the lamina ganglionaris, the medulla externa, and the medulla interna are absent. In the adult of *C. aeripennis destructor*, however, the optic centers are highly developed.

Deutocerebrum

The deutocerebrum (*2Br*) contains the antennal lobes, which are joined to one another by the deutocerebral commissure (*2Com*). Each antennal lobe gives rise to a single antennal nerve (*AntNv*), which passes to the anterior end of the brain before it emerges as a definitive nerve.

Tritocerebrum

The tritocerebrum (*3Br*) consists of a pair of lobes anterior to the deutocerebrum. The tritocerebral commissure (*3Com*), which connects the two lobes, is distinct from the circumesophageal connectives. The labral nerves (*LmNv*) and the frontal connectives (*FrCon*) arise from the tritocerebrum. The frontal connectives give rise to the stomodaeal nervous system.

Subesophageal Ganglion

The subesophageal ganglion (*SubGng*, Figs. 61, 63, 64) lies in the anterior part of the prothorax below the esophagus, and is the first ganglion of the ventral nerve cord. It is closely joined to the brain by the circumesophageal connectives (*CoeCon*, Figs. 63, 64). It innervates the mandibles, maxillae, and labium by the mandibular nerve (*MdNv*), the paired maxillary nerves (*MxNv*), and the paired labial nerves (*LbNv*) respectively.

The histological detail of the ganglion was not investigated. Its structure, as that of the other ganglia of the ventral nerve cord, consists of a central neuropile mass or medulla, with surrounding globuli cells or cortex. The histology of the subesophageal ganglion of insects has been little studied by precise neurological methods (32).

Nerves of the Head

The stomodaeal nervous system and the nerves innervating the labral region, antennae, and ocelli arise from the supraesophageal ganglion. The stomodaeal nervous system is described later.

The labral nerves (*LmNv*, Fig. 65) serve the nasale and the paranasal lobes. A true labrum is not present, hence there are no movable parts in this region and it is unlikely that the labral nerves have any motor fibers.

The antennal nerves (*AntNv*) give off some motor fibers which innervate the muscles of the antennae. They also contain the sensory fibers from the sensilla borne on the antennae.

The optic nerves (*OpNv*) branch shortly after they leave the supraesophageal ganglion. The anterior branches innervate the single pair of ocelli; each posterior branch (*TgNv*) innervates a region of the epicranium behind the ocellus. The posterior branches may innervate photoreceptors of some sort, but no receptors other than the ocelli were found.

The nerves serving the mandibles, maxillae, and labium arise from the subesophageal ganglion. The hypopharyngeal nerve of *Dissosteira carolina* (L.) also arises from this ganglion (32), but a nerve serving the hypopharynx was not found in the larva of *C. aeripennis destructor*.

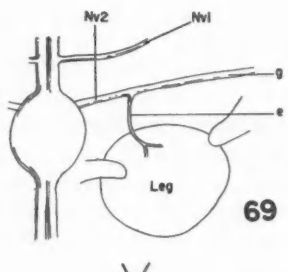
The mandibular nerves (*MdNv*, Fig. 68) arise as single nerves (Fig. 64) from the ventral side of the subesophageal ganglion, immediately behind the circumesophageal connectives. The single nerve branches after running cephalad a short distance. Each branch passes laterad where it breaks up into many finer branches, which innervate the mandibles and their muscles.

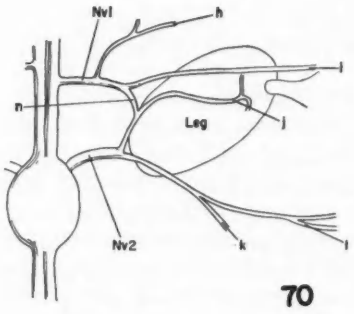
The maxillary nerves (*MxNv*, Fig. 68) arise from the ventral side of the subesophageal ganglion, behind the mandibular nerves. Each passes cephalad for some distance, branches, and passes laterad and cephalad to the maxilla. One branch, the nerve of the galea (*GaNv*), divides into two, to serve the galea and some of the muscles of the maxilla. The other branch divides into two; one nerve innervates the maxillary palp (*MxPlpNv*), the other innervates the lacinia (*LcNv*).

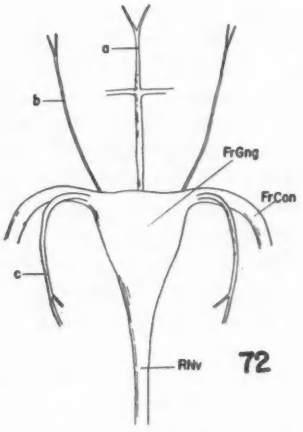
The labial nerves (*LbNv*) arise from the ventral side of the subesophageal ganglion behind the maxillary nerves and innervate the labium. They give off several small branches along their lengths which innervate the adductors of the labium.

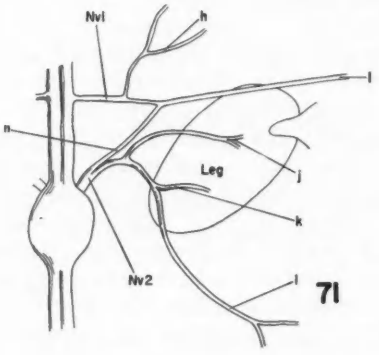
Ocelli

Descriptions of the external morphology of wireworms are numerous and in most cases (including Eidt (11)) the term "eye spots" is used. Glen (15) calls them eye spots in his description of *C. aeripennis destructor*. Arnason









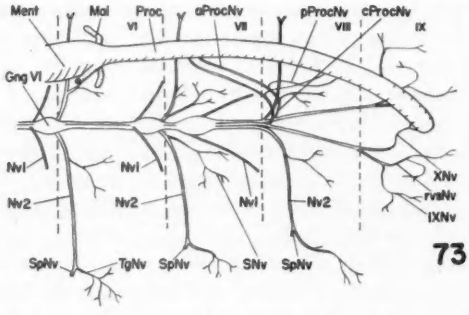


FIG. 69. Prothoracic nerves, dorsal. FIG. 70. Mesothoracic nerves, dorsal. FIG. 71. Metathoracic nerves, dorsal. FIG. 72. Frontal ganglion, dorsal. FIG. 73. Nerves of abdominal segments VI to X, dorsolateral.

(1) uses the term " 'eyes' ", and Henriksen (16) and Hyslop and Böving (19) call them ocelli. These photoreceptive organs in *C. aeripennis destructor* are true, although primitive, ocelli (Fig. 67). The overlying cuticle is transparent but not modified in shape, and forms the cornea (*Cor*). The epithelial cells which secrete the cornea are called the corneagen (*CgCls*). An oval crystalline body (*CB*) lies immediately under the corneagen. The sense cells (*SCls*) contain pigment granules and lie under the crystalline body. The axons of the sense cells continue as the optic nerve (*OpNv*) to the supraesophageal ganglion.

Ventral Nerve Cord

The ganglion of the prothorax (*Gng*1, Fig. 61) is located in the center of the segment, but the ganglia of the meso- and meta-thoraces are located in the anterior half of the segments. The ganglion of the first abdominal segment (*Gng*I) is located above the anterior conjunctiva of the segment, but subsequent ganglia are found farther cephalad until the ganglion of the seventh abdominal segment is almost completely within the sixth segment (Figs. 61, 73). The eighth abdominal ganglion (*Gng*VIII) is located in the center of the seventh abdominal segment.

From each of the segmental ganglia of the thorax of the abdomen of insects two or three principal lateral nerves proceed; and in some insects a median nerve arises posteriorly, or also anteriorly, between the bases of the connectives (32). In the larva of *C. aeripennis destructor* two lateral nerves arise from each ganglion. The median nerves are commonly present in larval forms (32) but they are absent in this larva.

Nerves of the Thorax

Two pairs of nerves innervate the prothorax. The first (*Nv*1, Fig. 69) arises from the sides of the connectives between the subesophageal ganglion and the prothoracic ganglion, and innervates the sternum. The second (*Nv*2) arises from the ventral side of the prothoracic ganglion and gives off short side branches (*e*) to the legs, then continues (*g*) to innervate the tergum.

Similarly, the mesothorax and metathorax are innervated by two pairs of nerves, but these nerves differ from those of the prothorax in the number of branches they give off. The first pair (*Nv*1, Fig. 70) arises, as in the prothorax, from the connectives immediately preceding the ganglion. Each one sends off a short branch (*h*) which innervates the sternum, then continues (*i*) to innervate the tergum. In the mesothorax the latter branch also innervates the spiracle. The second pair (*Nv*2) arises, as in the prothorax, from the ventral side of the ganglion. Each one sends off a branch (*j*) to innervate the leg, a branch (*k*) to innervate the sternum, and continues on (*l*) to innervate the tergum. Another nerve (*n*) connects the first and second nerves, but its position varies. For example, the other side of the mesothorax of the specimen drawn in Fig. 70 had an arrangement as in Fig. 71. Fig. 71 was drawn from the metathorax of the same specimen.

Nerves of the Abdomen

Each abdominal ganglion gives off two pairs of nerves. The first pair (*Nv*1, Fig. 73) arises from the sides of the anterior end of the ganglion and innervates the sternum. The second pair (*Nv*2) arises from the ventral side of the ganglion, and innervates the spiracles (*SpNv*), the sternum (*SNv*), and the tergum (*TgNv*).

The eighth abdominal ganglion, which is located in the seventh abdominal segment, is a composite of the ganglia of segments 8 to 10 and its nerves serve these three segments. The nerves of the eighth abdominal segment are homologous with those of the other abdominal segments, but are longer because the ganglion is remote from its segment. An additional pair of nerves passes cauded to innervate the ninth and 10th abdominal segments. They give rise to the common proctodaeal nerves (*cProcNv*) in the anterior part of the eighth abdominal segment. In the ninth segment, each divides to form the nerves of the ninth segment (IX*Nv*); the nerves of the 10th segment (X*Nv*); and the nerves of the retractor muscles of the 10th segment (*rvsNv*).

Visceral Nervous System

There are two parts to the visceral nervous system: the stomodaeal system and the proctodaeal system. Snodgrass (32) gives "visceral nervous system" and "stomodaeal nervous system" as synonymous, but Wigglesworth (37) considers the stomodaeal ("stomatogastric") system, the median nerves ("unpaired ventral nerves"), and the proctodaeal ("caudal sympathetic") system as parts of the visceral nervous system. The caudal sympathetic system, according to Wigglesworth, innervates the sexual organs and the posterior part of the gut. The larva of *C. aeripennis destructor* has no median nerves, as noted before, but it has a simple system of proctodaeal nerves which is considered here as a part of the visceral nervous system.

The frontal connectives (*FrCon*, Figs. 63, 64, 72) pass cephalad to the frontal ganglion (*FrGng*, Figs. 63, 64, 65, 72), which is the association center of the stomodaeal nervous system. The frontal ganglion lies on top of the pharynx (*FrGng*, Figs 6, 65) between the insertions of the dilatores buccales (*dlbc*) and the dilatores pharyngeales muscles (*dlphy*). It gives rise to two paired nerves and two unpaired nerves. The first (*a*, Fig. 72) is unpaired, arises from the anterior end of the ganglion, runs cephalad, and innervates the pharyngeal and cibarial muscles. The second (*b*) is paired, arises lateral to the first nerve, and also innervates the pharyngeal and cibarial muscles. The third (*c*) is paired, arises from the dorsal side near the ends of the frontal connectives, and innervates the dorsal suspensory muscles of the pharynx. The fourth nerve, the recurrent nerve (*RNv*) innervates the stomodaeum, and probably also the mesenteron and dorsal vessel. It is unpaired and is the largest of the four. It arises from the posterior end of the frontal ganglion and runs caudad along the dorsal side of the stomodaeum giving off branches to the stomodaeal muscles. The occipital ganglia, additional nerve centers of the stomodaeal system in some insects, are absent.

The proctodaeal nervous system arises from each of the pair of nerves of the ninth and 10th abdominal segments as the common proctodaeal nerves (*cProcNv*, Fig. 73). Each common proctodaeal nerve divides once to form the anterior proctodaeal nerve (*aProcNv*) and the finer posterior proctodaeal nerve (*pProcNv*).

Muscular System

The muscles of the head and those prothoracic muscles which control movements of the head are described here. The musculature of the digestive tract is described fully in the section on the digestive system and muscles associated with the spiracles are described in the section on the respiratory system. The alary muscles are described in the section on the circulatory system. The muscles which control the movements of the thorax, legs, and abdomen are not described.

The body muscles are of the common striated type with peripheral nuclei and they are surrounded by a delicate peritoneal tunic. The fibrillae occupy the whole cross section of the fiber; the nuclei are located immediately under the sarcolemma. This type of muscle is illustrated by Wigglesworth (37) in Fig. 62B. Muscles of the axial core type, with the nuclei in the center of the fiber, were not found. They are found in the adults of all the higher Diptera and Hymenoptera, according to Wigglesworth, and in the adult of *Dytiscus marginalis* (25).

Muscles of the Antennae

Three muscles (*mant*, Figs. 74, 75) control the movements of each antenna. They originate on the epicranium (*Epc*, Figs. 74, 76) near the socket of the anteriormost dorsosulcal seta (*dsse*, Fig. 76) and are inserted on long, slender apophyses (*apoant*) of the basal antennal segment. The center muscle is inserted dorsally and the other two are inserted on each side of the segment. Between them, the three muscles are able to control movement of the antenna in all directions.

Three muscles also control the antennae in the adults and in the larvae of *D. marginalis* (25). They are inserted on the basal segment of the antenna but originate on the tentorium rather than on the epicranium. Snodgrass (32) points out that the antennal muscles in some larval insects originate on the wall of the epicranium as they do in chilopods.

Korschelt distinguishes the antennal muscles of the larva of *D. marginalis* as an extensor, a flexor, and a levator (musculus extensor antennae, musculus flexor antennae, and musculus levator antennae). The antennal muscles of the larva of *C. aeripennis destructor* are probably homologous with those of *D. marginalis*. To use Korschelt's terminology, they are the levator (*lant*, Fig. 76), the extensor (*exant*), and the flexor (*flant*).

Muscles of the Labrum

The labrum of elaterid larvae is generally considered absent or fused with the clypeus and anterior part of the frons to form a "nasale", by morphologists who have studied the group (15, 4, 18, 31). Others (Roberts (28);

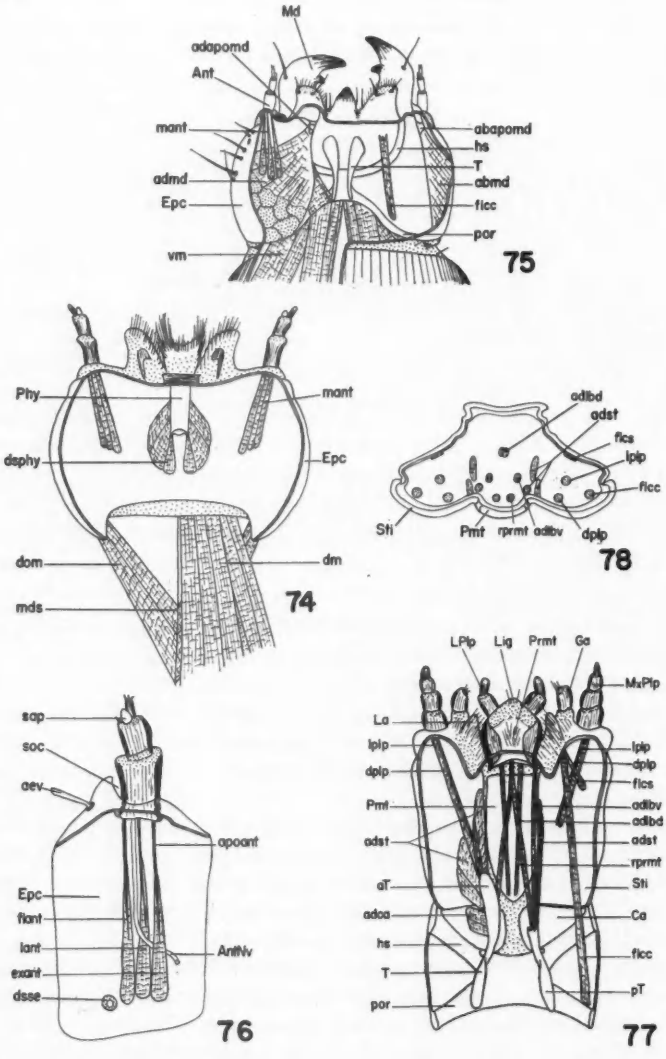


FIG. 74. Head, ventral, cut away to show muscles. Fig. 75. Head, dorsal, cut away to show muscles. FIG. 76. Antenna, ventral, showing muscles. FIG. 77. Ventral mouth parts, dorsal, cut away to show muscles. FIG. 78. Ventral mouth parts, cross section.

Horst (17); Znamenski (42)), suggest that the subnasale may be a rudiment of the labrum, but is not movable. Snodgrass (31) found that when the labrum is immovable on the clypeus, as it is in elaterid larvae, the muscles that control it are lacking. Das (7) states that the labrum is present in elaterid larvae (*Agriotes* sp.) and that it is drawn toward the epipharyngeal side. He states that the lateral labral muscles, which originate on the frons and insert on the tormae, are present. No labral muscles or tormae are present in *C. aeripennis destructor*, and examinations of *Agriotes lineatus* (L.) and *Agriotes mancus* (Say) do not confirm Das's observation. Dorsey (9) states that labral muscles are absent in the larva of *Parallelostethus attenuatus* and the larva of *Alaus* sp.

Muscles of the Mandibles

The mandible is controlled by two large muscles, the adductor and abductor. The adductor (*admd*, Fig. 75) is the largest muscle of the head. It originates on the side of the epicranium (*Epc*) and inserts on the adductor apophysis of the mandible (*adapomd*). One fascicle originates on the postgena near the tentorium. The adductor apophysis is a long, broad, flat membrane with a small sclerotized area near its base, and it is similar to that illustrated for *A. mancus* by Eidt (11, Fig. 36). The abductor (*abmd*, Fig. 75) is considerably smaller than the adductor. It originates on the side of the epicranium (*Epc*) ventral to the origin of the adductor and inserts on the abductor apophysis of the mandible (*abapomd*). The abductor apophysis is similar to the adductor apophysis but is narrower. The ventral adductor muscles, which are well developed in the Apterygota and sometimes present in pterygote insects as well (32), are absent. The adductors and abductors are the only mandibular muscles present in the larva of *D. marginalis* (25) but Korschelt calls them flexors and extensors respectively.

Muscles of the Hypopharynx

If the pharyngeal apophyses are the oral arms of the hypopharyngeal suspensoria, as suggested earlier, then the dorsal suspensors of the pharynx (*dsphy*, Figs. 74, 7) may be homologous with the retractors of the mouth angles. The retractors of the hypopharynx are absent.

Muscles of the Maxilla

The maxilla is not capable of lateral movements because it is fused with the labium, and its musculature is correspondingly modified. The entire unit formed by the fusion of the labium and maxillae is termed the "ventral mouthparts" by Glen (15) after Böving and Craighead (4). The dorsal muscles, the anterior and the posterior rotators, are absent. The ventral adductor is well developed and has its fibers separated into two groups, the adductor of the stipes (*adst*, Figs. 3, 4, 7, 77, 78) and the adductor of the cardo (*adca*, Figs. 5, 7, 77). Snodgrass (32) calls these muscles "adductors" because morphologically they correspond to the ventral adductors of a primitive appendage; their function in this insect is, however, to control the anterior

and posterior movements of the ventral mouth parts. Two fascicles of the adductor of the stipes insert well forward on the stipes and originate near the base of the tentorium, whereas the other fascicles originate on the anterior part of the tentorium. They are shown on the right half of Fig. 77. Snodgrass (31) calls this part of the adductor the third adductor of the stipes in describing the maxillary musculature of a caterpillar. It is probably the same muscle that Das (7) calls the retractor of the stipes.

The cranial flexor of the lacinia (*flcc*, Figs. 75, 77, 78) is well developed. It originates on the postgena and inserts at the base of the lacinia. This muscle does not belong to the endite lobes of the maxilla since, according to Snodgrass (32), the muscles of the terminal lobes of the maxilla always have their origins in the stipes. Snodgrass considers it homologous with the cranial flexor of the movable distal lobe of the diplopod mandible.

The stipital flexor of the lacinia (*flcs*, Figs. 77, 78) originates on the dorsal side of the stipes and inserts beside the cranial flexor of the lacinia. This is the only muscle of the endite lobes of the maxilla which is present.

Two muscles control each maxillary palp, a levator (*lplp*, Figs. 77, 78) and a depressor (*dplp*). Both muscles originate on the median basal part of the stipes and insert on the proximal margin of the basal segment of the palp.

Muscles of the Labium

The labium is considerably reduced from the generalized condition and its musculature is correspondingly reduced. Snodgrass (32) divided the muscles of the labium of generalized insects into four groups: those of the palps and terminal lobes; those inserted near the orifice of the labial glands; those extending from the postmentum to the prementum; and those extrinsic muscles which arise on the tentorium and insert on the prementum. The muscles of the first two groups are absent. One pair of muscles, the retractors of the prementum (*rprmt*, Figs. 8, 77, 78), extends from the postmentum to the prementum. They originate near the posterior end of the submentum and insert ventrally on the base of the prementum. The extrinsic muscles, the adductors of the labium are present. A pair is inserted dorsally (*adlbd*, Figs. 8, 77, 78) and a pair is inserted ventrolaterally (*adlbv*) on the base of the prementum as in generalized insects; both pairs originate on the anterior part of the tentorium (*aT*, Fig. 77). The labial muscles of the larvae of *P. attenuatus* and *Alaus* sp. as described by Dorsey (9) are the same. Das (7), on the other hand, described the same three pairs of muscles in the larva of *Agriotes* sp. but he called them the median, dorsal, and lateral muscles of the prementum respectively.

Prothoracic Muscles which Move the Head

Movements of the head are controlled by muscles which originate in the prothorax and insert on the posterior margin of the epicranium. The head is elevated by dorsal muscles (*dm*, Fig. 74) which originate on the conjunctiva between the prothorax and mesothorax. Lateral movements of the head are controlled by dorsal oblique muscles (*dom*) which originate along the

median dorsal suture (*mds*) of the pronotum. Ventral muscles (*vm*, Fig. 75), which oppose the dorsal muscles, depress the head and originate on the sternal apophyses of the prothorax and on the conjunctiva between the prothorax and mesothorax. All three of these muscles are present in the larva of *D. marginalis*, according to Korschelt (25). He calls them the musculus levator capitis horizontalis, musculus rotator capitis superior, and musculus depressor capitis horizontalis respectively.

Fat Body, Oenocytes, and Corpora Allata

The fat body, oenocytes, and corpora allata are considered together in this section, not because they have any structural or functional characteristics in common, but because none of them constitutes a "system" in the sense that the word has been used elsewhere in this paper.

Fat Body

The lobes of the fat body lie in the body cavity, secured in place by the tracheae which serve them. The bulk of the fat body (*Ft*, Fig. 80) surrounds the digestive tract (*Proc*) and is generally known as visceral fat body; parts of it extend between the muscles and the body wall and are known as peripheral or parietal fat body.

Snodgrass (32) states that the fat body has no definite organization, but Wigglesworth (37) says it is arranged in a constant manner in each species. In well-fed *C. aeripennis destructor* larvae the lobes appear to have a constant arrangement. The lobes of each segment are more or less distinct from those of the preceding and succeeding segments. In each segment there are five lobes. The first (*Ft*1) lies lateral to the digestive tract and is paired. It extends into the head (Fig. 79), it is found in the thorax and abdomen (Fig. 80) enveloping the lateral tracheal trunks, and it extends into the ninth abdominal segment (Fig. 81). It is shown in Figs. 82 and 83, which are dorsal dissections of the mesothorax and seventh abdominal segment respectively. A second lobe lies dorsal to the heart (*Ft*2, Figs. 80, 81). The third consists of paired lobes (*Ft*3, Fig. 80) which lie below the digestive tract, but above the ventral longitudinal muscles. In the ninth abdominal segment they lie on each side of the median genital capsule (*GenCp*, Fig. 81). The fourth lobe lies below the ventral nerve cord and below the ventral longitudinal muscles (*Ft*4, Figs. 80, 82, 83). The fifth lobe is paired, and lies between the lateral muscles and the body wall. In starved larvae the fat body recedes and the remnant surrounds the digestive tract, but it does not extend into the head nor into the ninth abdominal segment.

The fat body is composed of tightly packed polygonal cells which are bound together by an enveloping connective tissue membrane (Fig. 84). The fat cells are the largest cells in this insect, many being over 35 microns in diameter in well-fed larvae. The nucleus (*N*, Fig. 85) is about 7 microns in diameter. The cytoplasm is clear and scant, and contains large vacuoles (*va*) and many spherical eosinophilic globules (*gl*). In starved larvae the cells are smaller

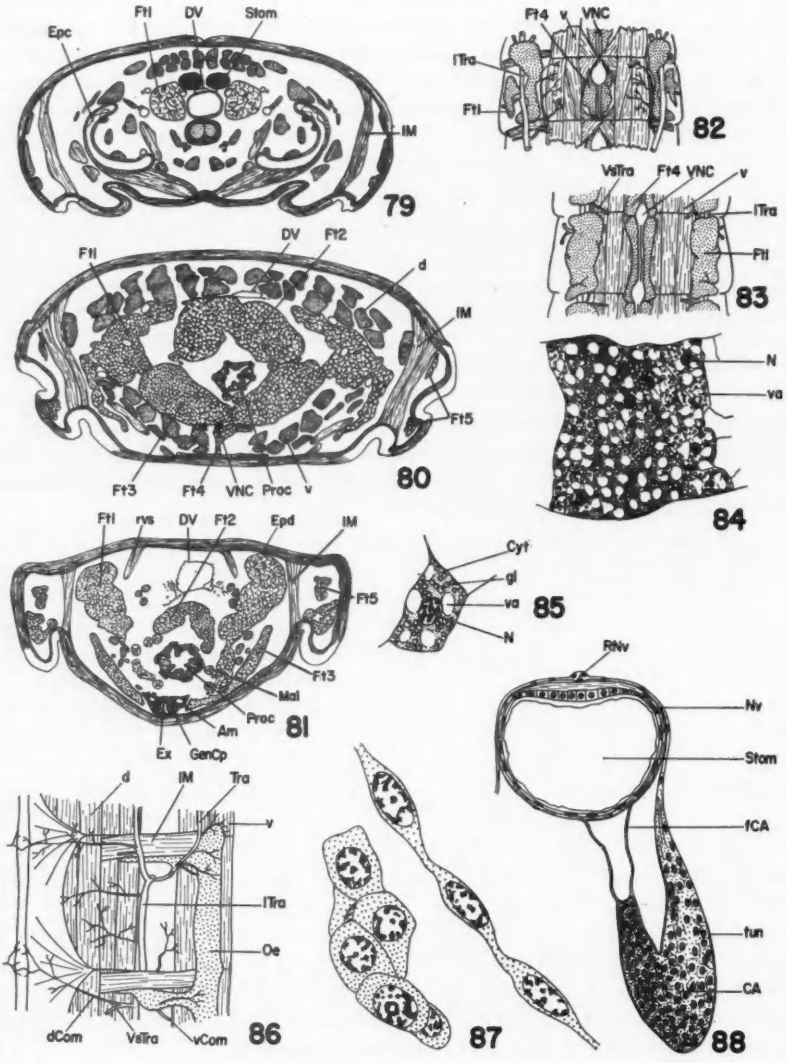


FIG. 79. Prothorax, cross section. FIG. 80. Seventh abdominal segment, cross section. FIG. 81. Ninth abdominal segment, cross section. FIG. 82. Mesothorax, dorsal view of ventral side. FIG. 83. Seventh abdominal segment, dorsal view of ventral side. FIG. 84. Fat body, cross section. FIG. 85. One fat cell. FIG. 86. Half of first abdominal segment, flattened out to show relation between oenocytes, muscles, and tracheae. FIG. 87. Oenocytes. FIG. 88. Corpus allatum, cross section.

and the vacuoles (*va*) and eosinophilic globules (*gl*) are larger and fewer in number. The vacuoles probably contained a fatty substance which was removed by the benzene used to clear the preparations.

Oenocytes

The oenocytes (*Oe*, Fig. 86) lie in a broad band, one cell thick, which lies between the fat body and the ventral longitudinal muscles (*v*). The band extends from the first to eighth abdominal segments, and ends just caudad to the eighth abdominal spiracle. Lobes of the oenocyte mass pass laterad and ventrad to the vicinity of the spiracles, and are intimately associated with the tracheae (*Tra*) and lateral muscles (*lM*). The oenocytes are similarly arranged in the larva of *D. marginalis* (25).

The oenocytes (Fig. 87) are not large cells although in most insects they are among the largest cells in the body. They measure about 9 microns in diameter, with nuclei about 5 microns in diameter, and are smaller than the fat cells and most of the blood cells (Fig. 87 is drawn to the same scale as the blood cells in Figs. 44 to 50). The nucleus is round or oval and has large distinct granules; the cytoplasm is hyaline.

Corpora Allata

The corpora allata (*CA*, Fig. 63) lie below the supraesophageal ganglion (*SupGng*) on each side of the stomodaeum (*Stom*). Each one (*CA*, Fig. 88) consists of a mass of compact, deeply staining cells surrounded by a thin connective tissue tunic (*tun*). Each corpus allatum is secured to the recurrent nerve (*RNv*) by a slender nerve (*Nv*) at one end; it loops mesad and is secured to the stomodaeum (*Stom*) by connective tissue fibers (*fCA*) at the other end.

The corpora allata are innervated from the occipital ganglia and invested in a sheath which is continuous with the neurilemma of the nerve (32). The occipital ganglia are absent in *C. aeripennis destructor* larvae and the corpora allata are innervated from the recurrent nerve. The tunic of each corpus allatum appears to be continuous with the neurilemma of the nerve, but, by definition, the term "neurilemma" cannot be applied to it.

Conclusions

The digestive tract is a simple tube with no loops or coils and it is about the same length as the larva. This conflicts with the general rule mentioned by Lewis (26) and Fletcher (13) that the digestive tract is longer in herbivorous insects than in carnivorous insects. Although many elaterid larvae are carnivorous, most are phytophagous (6) as is *C. aeripennis destructor*.

The pharynx forms a sucking pump, and the larva is unable to ingest solid particles (12). This is not how one would expect a mandibulate, phytophagous larva to feed. Only three other coleopterous larvae have been described as having sucking pumps, and two of these, *Dytiscus marginalis* (25) and *Lampyris noctiluca* (36), are predaceous forms with hollow mandibles. The third is the myrmecophilous beetle *Claviger testaceous* Preyssl.

The lack of openings from the spiracles into the tracheae may be of considerable importance in a consideration of the relationship of the insect to soil moisture. Such a condition has not been found in larvae of beetles other than elaterids.

Another interesting structural feature is the absence of median nerves and the innervation of the proctodaeum by a paired nerve from the last ganglion of the abdomen.

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ABBREVIATIONS USED IN FIGURES

a—a nerve of frontal ganglion.
*Ab*I–X—abdominal segments.
abapomd—abductor apophysis of mandible.
abmd—abductor muscle of mandible.
AcL—accessory lobe.
adapomd—adductor apophysis of mandible.
adca—adductor muscle of cardo.
adlbd—dorsal adductor muscle of labium.
adlbv—ventral adductor muscle of labium.
admd—adductor muscle of mandible.
adst—adductor muscle of stipes.
aev—ventral anteroepicranial seta.
am—alary muscle.
Am—ampulla.
An—anus.
Ant—antenna.
AntNv—antennal nerve.
aO—anterior margin of ostium.
Ao—aorta.
apoant—antennal apophysis.
aProcNv—anterior proctodaeal nerve.
aT—anterior part of posterior arm of tentorium.
Atr—atrium.

b—a nerve of frontal ganglion.
BM—basement membrane.
1*Br*—protocerebrum.
2*Br*—deutocerebrum.
3*Br*—tritocerebrum.

ABBREVIATIONS USED IN FIGURES—*Continued*

c—a nerve of frontal ganglion.
ca—calyx of corpus pedunculatum.
Ca—cardo.
CA—corpus allatum.
CB—crystalline body.
Cc—corpus centrale.
CgCls—corneagen.
cm—circular muscle.
CoeCon—circumesophageal connective.
1*Com*—protocerebral commissure.
2*Com*—deutocerebral commissure.
3*Com*—tritocerebral commissure.
Con—connective tissue.
Cor—cornea.
Cpd—corpus pedunculatum.
cphy—compressor muscle of pharynx.
cProcNv—common proctodaeal nerve.
Cpt—regenerative crypt.
Cv—corpus ventrale.
Cyt—cytoplasm.

d—dorsal longitudinal muscle.
*dCom*1–3, I–IX—dorsal tracheal commissures.
DDph—dorsal diaphragm.
dg—digestive cells.
dHdTra—dorsal head trachea.
dlbc—dilator buccalis.
dlcb—dilator cibarius.
dlphy—dilator pharyngealis.
dm—dorsal muscles of prothorax, controlling head.
dom—dorsal oblique muscles of prothorax, controlling head.
dph—diaphragm cells.
dplp—depressor muscle of maxillary palp.
DS—dorsal sinus.
dsphy—dorsal suspensor muscle of pharynx.
dsse—dorsosulcal seta.
dst—dorsal suspensor muscle of tentorium.
*dTra*1–3, I–VIII—dorsal tracheae.
*d*IX*Tra*—dorsal trachea of ninth abdominal segment.
DV—dorsal vessel.

e—a branch of second prothoracic nerve.
Epc—epicranium.
Epd—epidermis.
Ephy—epipharynx.
epr—epithelial ridge.
Epth—epithelium.
Es—esophagus.
Ex—exuviae of median genital capsule.
exant—extensor muscle of antenna.

f, *fa*, and *fb*—suspensory fibers of heart.
fCA—suspensory fiber of corpus allatum.
flant—flexor muscle of antenna.
flcc—cranial flexor of lacinia.
flcs—stipital flexor of lacinia.
FrCon—frontal connective.
FrGng—frontal ganglion.
Ft—fat body.
*Ft*1–5—lobes of fat body.
FtTra—trachea of fat body.

g—branch of second prothoracic nerve.
Ga—galea.
GenCp—median genital capsule.
GaNv—nerve of galea.
gl—fat globule.
*Gng*1–3, I–VIII—ganglia of ventral nerve cord.
GTra—trachea of gut.

h—branch of first nerve of meso- and meta-thoraces.
hr—branched hairs.
hs—hypostoma.
Ht—heart.

i—branch of first nerve of meso- and meta-thoraces.
In—intima.

j—branch of second nerve of meso- and meta-thoraces.

k—branch of second nerve of meso- and meta-thoraces.

l—branch of second nerve of meso- and meta-thoraces.
La—lacinia.
lant—levator muscle of antenna.
lbNv—labial nerve.
LcNv—nerve of lacinia.
*ld*IX*Tra*—laterodorsal ninth abdominal trachea.
Leg—leg.
LgTra$_{1-5}$—leg tracheae.
Lig—ligula.
lM—lateral muscles of abdomen.
lm—longitudinal muscle.
LmNv—labral nerve.
lplp—levator muscle of maxillary palp.
Lplp—labial palp.
Ltga—anterior laterotergite.
*Ltg*I–II—laterotergites.
lTra—lateral tracheal trunk.
Lum—lumen.

m—muscle.
mant—antennal muscle.
Mal—Malpighian tubule.
mds—median dorsal suture.
Md—mandible.
MdNv—mandibular nerve.
Ment—mesenteron.
Mth—mouth.
MxNv—maxillary nerve.
MxPlp—maxillary palp.
MxPlpNv—nerve of maxillary palp.

n–nerve joining first and second nerves of meso- and meta-thoraces.
N—nucleus.
Na—nasale.
Nlm—neurilemma.
IX*Nv*—nerve of ninth abdominal segment.
X*Nv*—nerve of 10th abdominal segment.

ABBREVIATIONS USED IN FIGURES—*Concluded*

*Nv*1—first segmental nerve.
*Nv*2—second segmental nerve.

Oc—ocellus.
Oe—oenocytes.
OpL—optic lobe.
OpNv—optic nerve.
OpT—optic tract.
or—orifice of spiracle.

p—trabeculated septum.
PCls—pericardial cells.
PcrGb—protocerebral globuli cells.
PcrL—protocerebral lobe.
pd—pedunculus of corpus pedunculatum.
pdd—dorsal root of pedunculus.
pdm—median root of pedunculus.
Phy—pharynx.
phya—pharyngeal apophysis.
Picr—pars intercerebralis.
Pmt—postmentum.
Pn—paranasal lobe.
pO—posterior margin of ostium.
por—postoccipital ridge.
pProcNv—posterior proctodaeal nerve.
Prmt—prementum.
Proc—proctodaeum.
pT—posterior part of posterior tentorial arm.
Ptr—peritreme.

q—thin place in intima of atrium of spiracle.

r—ridge of peritreme of spiracle.
rg—regenerative cells.
*rm*1–4—rectal muscles.
RNv—recurrent nerve.
rprmt—retractor of prementum.
rvs—retractor muscles of 10th abdominal segment.
rvsNv—nerve of retractor muscles of 10th abdominal segment.

sap—sensory appendix of antenna.
sb—striated border.
SCls—sense cells.
SNv—nerve of sternum.
soc—ventral socket of mandible.
SpC—spiracular chamber.
SpNv—spiracular nerve.
SpSc—spiracular sclerite.
*SpTra*1–3, I–VIII—spiracular tracheae.
Sti—stipes.
Stom—stomodaeum.
SubGng—subesophageal ganglion.
SupGng—supraesophageal ganglion.
SVlv—stomodaeal valve.

t—closed primary spiracular orifice.
T—tentorium.
TgNv—tegumentary nerve.
*Th*1–3—thoracic segments.
2ThSp—mesothoracic spiracle.
3ThSp—rudiment of metathoracic spiracle.
Tn—taenidia.
Tra—trachea.

X*Tra*—trachea of 10th abdominal segment.
tun—connective tissue tunic.

v—ventral longitudinal muscle.
va—vacuole.
*vCom*1–3, I–VIII—ventral tracheal commissures.
vComHd—ventral commissure of head.
vHdTra—ventral head trachea.
Vm—ventral mouth parts.
vm—ventral muscles of prothorax, controlling head.
VNC—ventral nerve cord.
vsphy—ventral suspensor muscle of pharynx.
VsTra—visceral trachea.
*v*IX*Tra*—ventral trachea of ninth abdominal segment.

EFFECTS OF ACCLIMATIZATION AND SEX ON RESPIRATION AND THERMAL RESISTANCE IN TRIBOLIUM (COLEOPTERA:TENEBRIONIDAE)[1]

D. K. EDWARDS[2]

Abstract

Some effects have been studied of prolonged exposure to nonoptimal temperatures on respiration and thermal resistance in the flour beetle *Tribolium confusum* Duval.

Adult beetles maintained at 18° C. and 38° C. for a number of months exhibited changes in the location and form of the temperature–respiration curve compared with that of control insects at 30° C. Insects from 18° C. showed increased survival ability at −3° C., but there was no respiratory compensation during acclimatization. Changes in the sign of respiration–weight regression in the experimental groups was probably related to differences in sensitivity to temperature between lighter and heavier individuals.

Control 30° males survived exposure to −3° C. better than corresponding females. This survival difference at the low temperature was eliminated by prolonged maintenance of the insects at 18° C. Insects from 18° C. possessed a higher water content and a lower dry weight than controls. A discussion is presented dealing with the influence of the ratio of active to inactive weight on respiration per unit weight.

The temperature–respiration curves were adequately expressed in the double-logarithmic form. The constants in this equation were used comparatively among the different experimental groups.

Introduction

Previous temperature history is one of the most important factors influencing the nature of the metabolic response of an insect to temperature (30). Acclimatization to cold occurs in insects, but the evidence for experimental heat acclimatization in insects is still not great.

Robinson (29) carried out studies of acclimatization to cold in various insects and showed that they can adapt. Mellanby (19), in studies of cold adaptation in various insect genera, showed that those from a warm environment were less cold-hardy than those from a cooler environment. Adjustment to a lower temperature, measured by activity, occurred in these insects within twenty hours. This same author (20) found in some species of Arctic insects that the stages normally exposed to cold for long periods were less immobilized by temperatures down to 0° C. than stages which were normally exposed to a warm environment. Whitney (36), working with May fly nymphs from ponds and streams, found that those from slow or still waters possessed greater heat tolerance than those from swift streams. He related the greater thermal adaptation to greater temperature fluctuations in the undisturbed environment. Walshe (35) found that larvae from streams below 15° C. were less resistant to high temperatures than larvae from still waters at 20° C. Baldwin (2) and Baldwin and House (3) increased the thermal

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resistance of sawfly larvae by thermal conditioning. Colhoun (9) acclimatized *Blatta* to cold, thermal conditioning being evidenced by a greater activity at low temperatures.

Agrell (1) investigated the influence of previous temperature history on a number of insects which had undergone thermal adaptation over a period of weeks. Insects were transferred from their normal environmental temperature to 5°, 18°, and 30° C. The respiration of each group was measured at 18° C. All experimental insects showed a quantitative change in gas consumption with changes in the previous temperature history. Agrell stated that insects may respond to temperature changes either with or without respiratory compensation.

Bullock (7) concluded that there are general indications of poor compensatory adjustment to temperature changes in insects. However, Dehnel and Segal (11) and others have shown that some insects exhibit respiratory adaptation at different temperatures.

The present work represents a portion of a study of various factors which affect the respiration and thermal resistance of *Tribolium confusum* Duval, the confused flour beetle (12). It was intended to establish first a temperature–respiration curve for male and female normal *T. confusum* reared at 30° C. These curves could be used as controls for comparison with curves obtained from insects with different previous temperature histories. A comparison between control insects and those with different previous temperature histories could also be made on the basis of survival at extreme temperatures.

A preliminary report on some of the results from these experiments has already been made (13).

Methods

Adult *T. confusum* were used in all the experiments. Cultures of this species have been maintained at McGill University for over 10 years. These cultures are descendants of original stock used by R. N. Chapman for his work on the biotic constants of this insect (8). The cultures were therefore quite uniform genetically. The food medium was whole-wheat flour with 3% ground wheatgerm added. The cultures were maintained at 30° C., the physiological optimum (14, 34), and at a relative humidity of 75%.

For sexing the insects, the method of Hope (16) was used. This method is based upon the pattern of the lines engraved on the elytra. Well in excess of 100 live *T. confusum* can be sexed in 1 hour using this technique.

Some insects were taken from the 30° control culture as 2-months-old adults and maintained in constant temperature cabinets at 18° C. and 38° C., relative humidity 75%. They were kept at these temperatures for approximately six months prior to their being used in experiments.

Respiration was measured over a series of temperatures with Barcroft respirometers. A single sample consisted of 10 insects of one sex. The sample was put into the appropriate respiration chamber of the respirometer with small strips of flour-paper (10). The cup, with the insects, was allowed

to stand overnight in the constant-temperature cabinet from which the insects had just been taken. This "waiting period" was found to be necessary in order to allow the insects to recover fully from handling effects. The following day the oxygen consumption was determined at the temperature to be investigated. The insects remained quiescent in the respiration chamber during the experiment. Readings were taken until the manometer deflection assumed a constant value. The sample of insects was then removed and weighed, and the oxygen consumption per milligram determined. Thus, one point was established on the temperature–respiration curve under investigation. Five such points were obtained at each experimental temperature for each sex.

Survival at −3° C. and +40° C. was investigated for 30° control insects and insects maintained at 18° C. and 38° C. One-liter glass museum jars were used to contain the insects at the experimental temperatures. Samples of experimental and control insects were placed in the jars together with a small dish of a saturated salt solution to maintain the relative humidity as close to 75% as possible.

Control insects which had been reared and maintained at 30° C. will henceforth be referred to as 30° insects. The two experimental groups will be referred to as the 18° and the 38° insects.

Observations

A. Respiration

The values for oxygen consumption per unit weight for control 30° insects and for both 18° and 38° insects are given in Table I with 95% confidence limits. Only females were investigated in the 38° group. Each value for oxygen consumption represents the mean of five determinations. An overlapping of the confidence limits of two comparative values (i.e. male and female values at the same temperature, or values for the control and the experimental groups at the same temperature and of the same sex) is taken to indicate no significant difference.

TABLE I

MEAN VALUES FOR OXYGEN CONSUMPTION AT VARIOUS TEMPERATURES (MM.³/MG./HR.) FOR 30° CONTROL, 18°, AND 38° *T. confusum*, WITH 95% CONFIDENCE LIMITS

	30°		18°		38°
T.	♂	♀	♂	♀	♀
5°	0.160±0.073	0.184±0.052			
10°	0.317±0.023	0.338±0.038	0.165±0.060	0.211±0.123	0.179±0.050
18°	0.808±0.197	1.08 ±0.14	0.450±0.056	0.703±0.176	0.745±0.133
26°	1.54 ±0.31	1.70 ±0.04			
34°	2.63 ±0.67	2.77 ±0.21	1.97 ±0.71	2.29 ±0.51	2.21 ±0.57
40°	3.54 ±0.43	3.31 ±0.06	2.90 ±0.72	3.24 ±0.52	2.88 ±1.18
44°	3.80 ±0.78	4.29 ±0.46	3.63 ±0.50	4.02 ±0.78	3.84 ±0.47

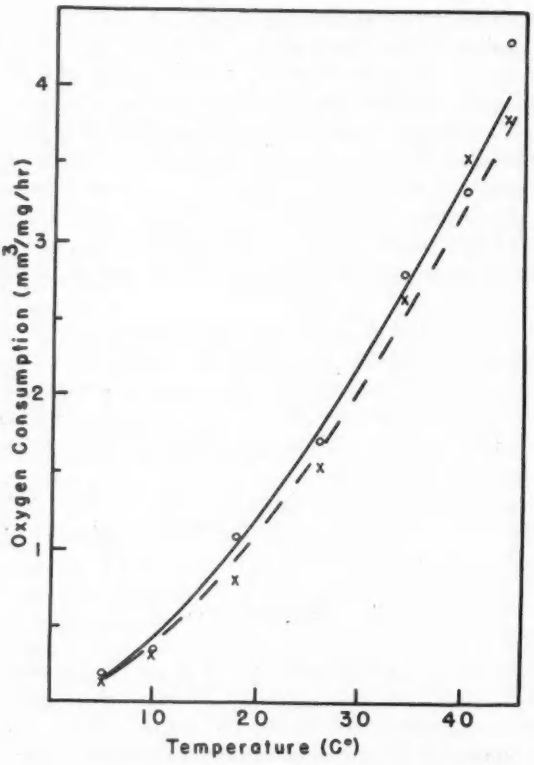


FIG. 1. T–R curves for male (broken) and female (solid) control 30° *T. confusum.* Each point near the lines indicates the mean of five respiration determinations (open circles for females).

The temperature–respiration curves for male and female 30° control *T. confusum* are illustrated in Fig. 1. Points near the curves indicate the mean values given in Table I. The lines of best fit were calculated by the method of least squares. A straight line resulted when the temperature–respiration data were plotted on a double-logarithmic grid. The following equation was therefore considered to be the most convenient way in which to express the data:

$$\log R = \log a + b \log T$$

where R represents the vertical axis, or oxygen consumption; T represents the horizontal axis, or temperature; a and b are constants. The equations of the lines of best fit for 30° males and females are:

Males: $\log R = -1.968 + 1.540 \log T$
or $R = 0.011\ T^{1.54}$

Females: $\log R = -1.855 + 1.490 \log T$
or $R = 0.014\ T^{1.49}$

An F test for goodness of fit of the data, with both variables in logarithmic form, indicated that 97.2% of the variation in log R in males was accounted for by regression of log R on log T. Similarly, 98.2% of the log R variation in females was accounted for by regression on log T. In both cases this may be considered a good fit of the log–log data to a straight line (33).

A comparison of the temperature–respiration curves of female 30°, 18°, and 38° *T. confusum* has already been reported (13). A table was presented in that report showing the values of the constants in the T–R curves together with 95% confidence limits. Table II in the present work includes data from that report and is extended to include the constants for males. Fig. 2 has been taken from the previous work. The temperature–respiration curves of female 30°, 18°, and 38° *T. confusum* are compared in semilogarithmic form, illustrating changes in Q_{10} over the temperature range. The Q_{10} is reflected by the slope of the tangents to the curves.

TABLE II

VALUES FOR T–R EQUATION CONSTANTS, WITH 95% CONFIDENCE LIMITS AND RANGES, FOR 30° CONTROL, 18°, AND 38° *T. confusum*

		log a		b	
Group	Sex	Constant	Range (a)	Constant	Range (b)
18°	Males	−2.959±0.199	0.000694–0.00174	2.124±0.169	1.95–2.29
18°	Females	−2.707±0.204	0.00123 –0.00314	2.008±0.174	1.83–2.18
30°	Males	−1.968±0.125	0.00808 –0.0143	1.540±0.092	1.45–1.63
30°	Females	−1.855±0.100	0.0111 –0.0175	1.490±0.072	1.42–1.56
38°	Females	−2.717±0.151	0.00136 –0.00271	2.002±0.128	1.87–2.13

Calculation of the 95% confidence limit ranges for the constants in Table II shows that corresponding values for males and females do not differ significantly in either the 30° control group or the 18° group. Comparison of the constants for the same sex between the control and the experimental groups reveals significant differences. The values for a in both the 18° and the 38° groups are lower than the a value for the corresponding sex in the 30° controls. The values for b in the experimental groups are greater than that for the corresponding sex in the 30° controls.

Although the curve for males is depressed below that for females in Fig. 1, comparison of the values for oxygen consumption at any experimental temperature along the curves reveals no significant difference.

In Fig. 2 the T–R curves for 18° and 38° females are depressed below that for 30° females. On the basis of comparison of the constants a in the curve equations this depression is statistically significant. The significantly greater b in both the 18° and 38° curves compared with control curves is reflected in the greater curvature of the former. Thus, at any given temperature along the curve the Q_{10} in the 18° and 38° groups is greater than that of 30° controls.

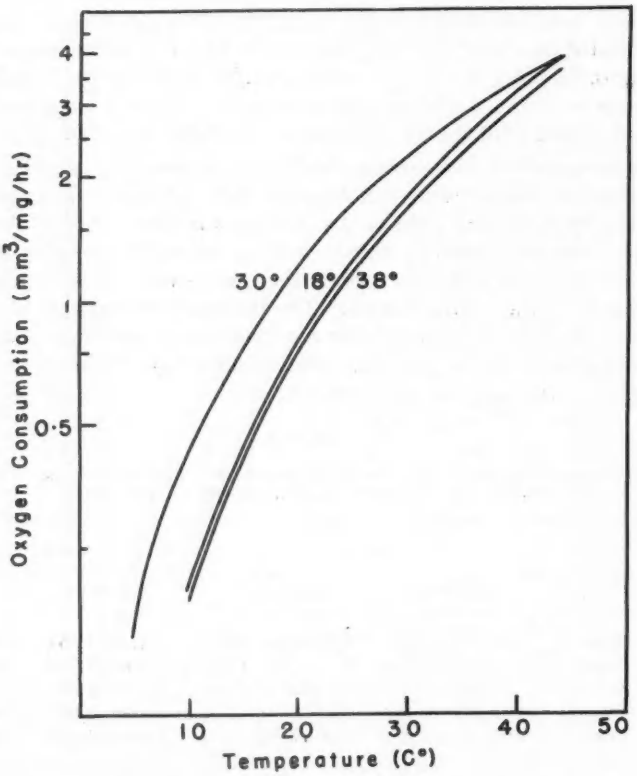


FIG. 2. Semilogarithmic plot of T–R curves of female *T. confusum* from 18° C., 30° C., and 38° C.

B. Survival at Extreme Temperatures

The results of exposure of 30° control, 18°, and 38° *T. confusum* to +40° C. for various times are given in Table III. Males and females are not treated separately, since no sex difference in survival ability was found at the high temperature. The LD_{50} 95% confidence limits for each of the three groups exposed to 40° C. are given in Table IV.

Tables III and IV show that survival at 40° C. in the 18° insects was greater than that of 30° controls. Survival in the 38° group was less, compared with that of the controls. The statement of significance is based upon non-overlapping of the LD_{50} 95% limits.

A sample of 30° control insects in each of the two experiments wherein 38° insects were exposed to 40° (4 and 5 days respectively) was used in a direct Chi^2 comparison with the latter to test for a difference in survival. In both the 4 and 5 day experiments the survival of the 38° insects was significantly lower than that of the corresponding controls. This is in agreement with the difference between the LD_{50} values of the two groups.

A Chi² test was made to compare two groups of 18° and 30° control insects kept for 4 days at 40° C. A significant difference in survival was apparent between 18° insects and 30° controls after 4 days at 40° C. There was a greater survival in the former group. This is in agreement with the difference between the two LD_{50} values.

TABLE III

PER CENT SURVIVAL OF 30° CONTROL, 18°, AND 38° *T. confusum* DURING EXPOSURE TO +40° C. VALUES FOR DIFFERENT TESTS ARE GIVEN, WITH MEAN PER CENT SURVIVAL IN PARENTHESES; $n=50$ IN EACH TEST

Exposure time, days	Per cent survival				
	30°		18°		38°
4	48 54 46	(49.3)	62 96	(79)	24
4.8	38				
5	28 34 36 38	(34)	76		10
6	16		18		

TABLE IV

NINETY-FIVE PER CENT CONFIDENCE LIMITS FOR LD_{50} VALUES FOR THREE TEMPERATURE GROUPS EXPOSED TO −3° C. AND +40° C.

		−3° C.	+40° C.
Group	Sex	LD_{50} limits, hr.	LD_{50} limits, days
30°	Male	42.61– 46.53	3.72–4.34
30°	Female	27.53– 38.98	
18°	Male	86.28–107.26	5.09–5.61
18°	Female	81.13– 96.39	
38°	Male	31.75– 50.40	0.81–1.66
38°	Female	22.57– 33.60	

The mortality trends in both sexes of 30°, 18°, and 38° *T. confusum* exposed to −3° C. are indicated in Fig. 3. Males and females are treated separately. Percentage mortality values were converted to probits and plotted against time at the experimental temperature. The intersection point of the line at the probit value 5.0 represents the LD_{50}. Points near the lines indicate mortality values based upon a mixed-sexes sample of 50 insects at each exposure time.

The values for the LD_{50} were calculated by fitting a straight line to the mortality percentages converted to probits and solving the equation of the line

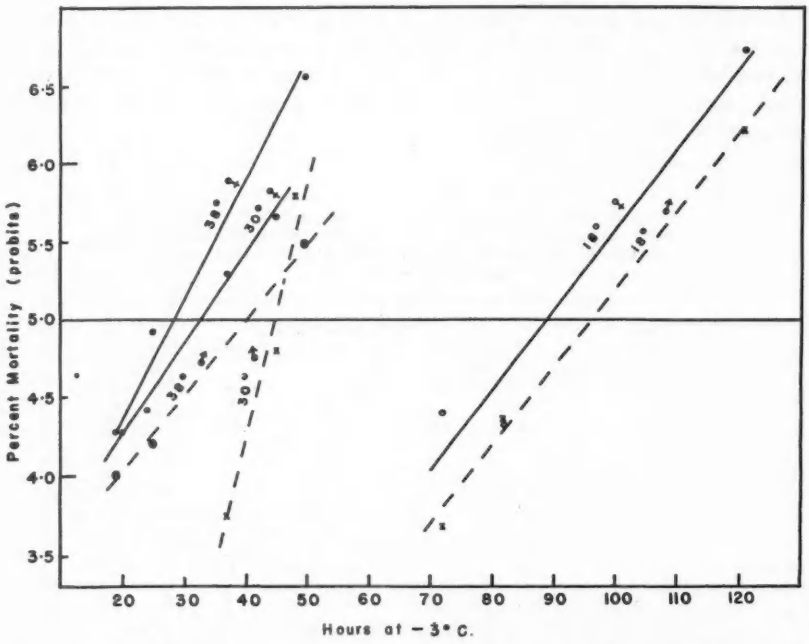


FIG. 3. Mortality in both sexes of *T. confusum* from three different temperatures after exposure to −3° C. Points near the lines indicate mean values for each sample.

for x (time) with y equal to 5.0. Confidence limits were placed about the LD_{50} values by the method of Litchfield and Wilcoxon (18). The 95% confidence limits for the LD_{50} values are given in Table IV.

The survival of male and female 30° controls after 24, 45, and 48 hours at −3° C. were compared by means of a Chi^2 test to determine whether a survival difference existed between the two sexes. The Chi^2 diagram is shown in Table V. The calculated Chi^2 is 4.4, while the chance value for Chi^2 is 3.8 (d.f. 1).

The Chi^2 test shows a significant difference in the survived–dead ratio between 30° control males and females at −3° C. The survival of males is greater, under the conditions of the experiment. This is in agreement with the smaller LD_{50} in 30° females shown in Table IV.

Both sexes of *T. confusum* kept for some time at 18° C. showed a significantly increased survival ability when compared with that of 30° controls (Table IV). In addition, prolonged exposure to 18° C. eliminated the sex difference in survival at −3° C. This is revealed by comparison of the two LD_{50} confidence limits.

Prolonged exposure of *T. confusum* to 38° C. did not significantly alter the LD_{50} at −3° C. compared with the same sex in the 30° control group. However, as was the case in the 18° insects, no sex difference in survival ability at the low temperature was apparent in the 38° insects.

TABLE V

NUMBER OF SURVIVING AND DEAD 30° CONTROL *T. confusum* AFTER 24, 45, AND 48 HOURS AT −3° C. THE TOTAL NUMBER OF INSECTS AT EACH EXPOSURE TIME IS 50

	Survived	Dead	Σ
Males	26	3	
	15	11	
	7	26	
	48	40	88
Females	15	6	
	6	18	
	2	15	
	23	39	62
Σ	71	79	150

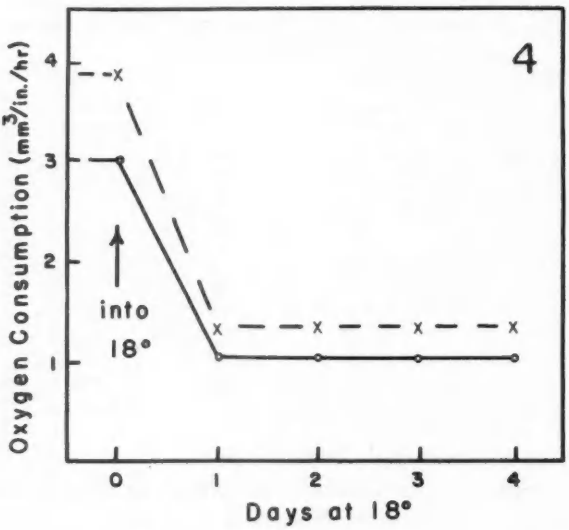
C. Early Effects of Nonoptimal Temperatures

Some experiments were carried out to determine the nature of the changes in oxygen consumption during the first few days of continual exposure of 30° control *T. confusum* to 18° C. and 38° C., relative humidity 75%. The oxygen consumption of a sample of 10 insects was first measured at 30° C., the control temperature. The insects were then transferred to either the high or low experimental temperature in the respirometer chamber. Further respiration determinations were made at daily intervals at the experimental temperature.

The results of the above experiments are shown in Figs. 4 and 5. Fig. 4 shows the change in the respiration of both sexes during exposure to 18° C. Fig. 5 illustrates the changes which occurred during exposure to 38° C. Oxygen consumption is expressed in cubic millimeters per insect per hour.

The respiration–time curves in Fig. 4 show that the oxygen consumption reacted passively to the lowered temperature, showing no respiratory compensation.

In Fig. 5 the oxygen consumption of both sexes increased immediately following exposure to 38° C. This increase corresponds to the change in respiration level along the temperature–respiration curve of control insects (Fig. 1). In males the oxygen consumption rose to a maximum 1 day after exposure to 38° C. In females the maximum respiration was reached in 2 days. The oxygen consumption fell beyond the maximum in both sexes. In males the "overshooting" of the respiration lasted 2 days. The respiration in males returned to a final level which was higher than that immediately following exposure to 38° C. In females the period of "overshooting" lasted 3 days and the respiration returned to the level of that immediately following initial exposure to 38° C.



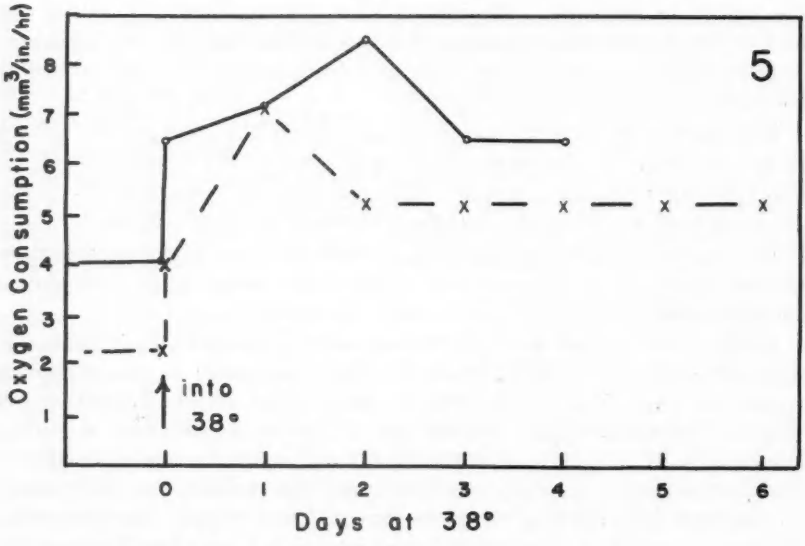


FIG. 4. Change in respiration of a sample of 10 male (broken line) and one of 10 female (solid line) 30° *T. confusum* during exposure to 18° C.

FIG. 5. Change in respiration of a sample of 10 male (broken line) and one of 10 female (solid line) 30° *T. confusum* during exposure to 38° C.

D. Weight

(i) Sex Differences

The wet weights of all samples of 30° control *T. confusum*, used to calculate the oxygen consumption per milligram, were recorded and the data analyzed to test for a possible difference in weight between the sexes.

A total of 60 samples (10 insects each) of males gave a mean weight of 20.01 mg. per sample. For females, a total of 55 samples gave a mean weight of 23.11 mg. per sample. The difference in weight is highly significant.

(ii) Respiration–Weight Regression

The five oxygen consumption readings at each temperature for both sexes of control 30° *T. confusum* are plotted against corresponding weights for the samples in Fig. 6. In some cases, for example in males at 34° C. and 44° C.,

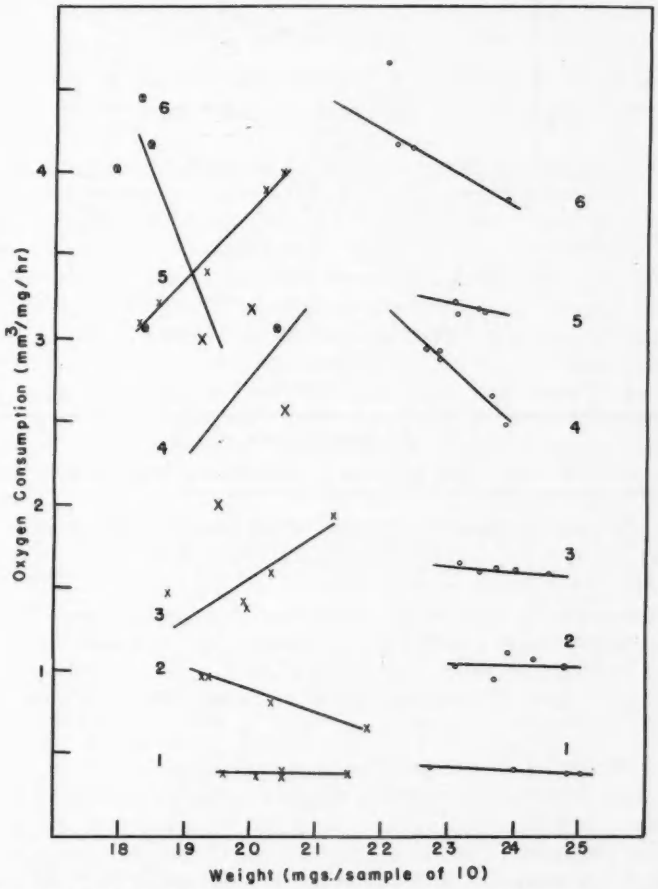


FIG. 6. Respiration–weight regression at different experimental temperatures for male (smaller weight) and female 30° *T. confusum*. 1, 10°; 2, 18°; 3, 26°; 4, 34°; 5, 40°; 6, 44°.

the scatter of the points is fairly large. Thus, the slope of the line indicated in these cases is not too reliable.

The sign of regression is essentially opposite in males and females at each temperature except 18° C. and the upper extreme. In females, increased weight is correlated with a lower oxygen consumption at each experimental temperature, while in males the opposite is true. In both sexes respiration is less size dependent at low temperatures. In females, there is a decreasing Q_{10} with increasing weight except between 34° C. and 40° C. Lighter females were more sensitive to temperature change. In males, there is an increasing Q_{10} with increasing weight between 18° C. and 26° C., 26° C. and 34° C., and between 26° C. and 40° C. Heavier males were more sensitive to temperature change.

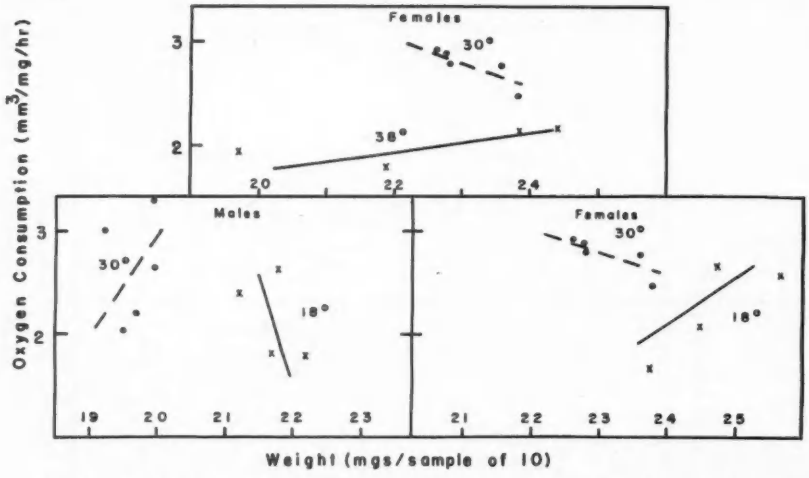


FIG. 7. Respiration–weight regression of 18° male and female, and 38° female *T. confusum* compared with that of 30° insects; measured at 34° C.

The effect of long-term maintenance of the insects at the nonoptimal temperatures on respiration–weight regression is shown in Fig. 7. The temperature 34° C. was chosen since data from the T–R curves could be used, and this temperature is close to the optimum. Prolonged exposure to the nonoptimal temperatures resulted in a reversal of the regression sign. Lighter females and heavier males were affected most. This sensitivity difference among individuals of different weights is in agreement with the situation shown in Fig. 6.

(iii) Weights of Nonoptimal-Temperature Groups

Menusan (21) reported that the weight of adult *T. confusum* increased as the temperature of the environment decreased, with food moisture and relative humidity constant. The wet weights of both 18° and 38° insects in the present work are compared with values for 30° controls in Table VI. Weights were based upon samples of 10 insects.

TABLE VI

WEIGHTS OF SAMPLES OF 30° CONTROL, 18°, AND 38° *T. confusum*

Group	Sex	Weight, mg.	No. of samples	Sum of squares	Variance
30°	Males	20.01	60	75.62	1.28
30°	Females	23.11	55	137.79	2.55
18°	Males	21.36	22	8.56	0.41
18°	Females	24.58	28	14.18	0.53
38°	Females	22.18	25	34.94	1.46

The weight of 18° insects, males and females, is significantly higher, and that of 38° females significantly lower, than the weight of controls of the same sex.

(*iv*) *Water Content*

A test was made to determine whether the difference in weight between control and 18° insects was related to possible differences in the water content of the insects.

The wet weights of samples of 30° control insects, consisting of 34 males and 34 females, were determined. The insects were then placed in a vacuum desiccator at 60° C. for 2 days. The dry weight was then determined. Table VII gives the wet and dry weight of 30° and 18° insects in two tests.

Although the 18° insects possessed a wet weight greater than that of the controls, the dry weight of 18° insects was less than the dry weight of control insects. The greater weight of 18° insects originally found seems to have been due, therefore, to a greater water content compared with 30° controls. The relative increase in water was sufficiently great to mask the decreased dry weight in 18° insects. Table VII reveals that the greater wet weight of females compared with males is reflected in a greater dry weight in the former.

TABLE VII

WET AND DRY WEIGHTS (MG.) OF BOTH SEXES OF 30° CONTROL AND 18° ADAPTED INSECTS FROM TWO TESTS; $n=34$

	30°			18°		
	Test	I	II	Test	I	II
Males	Wet	75.74	73.03	Wet	76.71	76.70
	Dry	39.80	36.87	Dry	33.97	33.22
	Water	35.94	36.16	Water	42.74	43.48
	% water	47.5	45.5	% water	55.7	56.7
Females	Wet	84.01	79.25	Wet	89.87	87.10
	Dry	46.83	40.73	Dry	38.82	37.84
	Water	37.18	38.52	Water	51.05	49.26
	% water	44.3	48.6	% water	56.8	56.6
Average % water, male and female		45.9	49.0		56.2	56.7

Discussion

A. Respiration

The fitted temperature–respiration curves for 30° males and females, when extrapolated, pass through the origin. It does not follow from this, however, that the respiration of the insects necessarily becomes zero at 0° C. The curve is described as being a good fit between the experimentally determined limits only. Respiration is maintained at a very reduced degree into the subzero temperatures. It has been shown that the insects may survive −3° C. for a short time.

Park (24) reported that female *T. confusum*, in his experiments, consumed significantly more oxygen than males per unit weight. His results were based upon probable error.

Saakyan (31) reported that the "breathing intensity" of *T. confusum* males is higher than that of females, especially in imagoes. No statistical analysis of the results was presented.

The present work has not shown a significant difference in oxygen consumption per unit weight between the sexes at each experimental temperature. There was no sex difference in the two constants of the T–R equations. There does exist, however, a *trend* in the direction of a higher oxygen consumption in females over the whole temperature range, in tentative agreement with Park. This is indicated by the higher value and confidence limits of a in both 30° females and 18° females compared with corresponding males. Female insects weighed more than males. On the basis of the "surface rule" (26) females might be expected to show less oxygen consumption per unit weight compared with males if the former were larger. However, there is no evidence that females were larger. The observed difference in respiration between the sexes may have been related to the sex difference itself, as originally suggested by Park, or to relatively more respiring tissue in females.

No definite reason can be given at this time for the differences in respiration per unit weight between lighter and heavier individuals of the same sex. However, some speculation on this point might be of value. Larger insects would possess proportionately less cuticle compared with body volume, as a result of a smaller relative surface area. In larger insects the *relative* decrease in "inactive" cuticle, compared with the volume of respiring tissue, would tend to increase the value obtained for oxygen consumption on a unit weight basis. This would aid in explaining the increased oxygen consumption in larger 30° males compared with smaller ones. The greater oxygen consumption would also be facilitated if the greater weight per se was related to a greater amount of more active tissue (for example, sperm).

In 30° females the lower oxygen consumption per unit weight in heavier individuals is in apparent agreement with the "surface rule", if "heaviness" was related to size. This trend toward decreased oxygen consumption would be opposed by the relative decrease in the amount of "inactive" cuticle. However, there is no evidence that "heaviness" was related to size. A greater mean weight in some females compared with others may reflect the weight of

eggs in the former. More eggs would mean a greater relative "inactive" weight, due to an increased amount of stored food, and would tend to lower the oxygen consumption per unit weight. Bodine (6) has reported that the greater weight in female *Dichromorpha* (Orth.) compared with males is due to the weight of eggs in the former.

To summarize these points, the trend toward a greater oxygen consumption in females, compared with males, may be related to a greater relative amount of respiring tissue in the former. The decreased oxygen consumption per unit weight in heavier females, compared with lighter ones, may have been related to a greater abundance of eggs in the former. The greater respiration in heavier males, compared with lighter ones, may have been related to relatively more respiring tissue in the former.

The depression of both the high- and low-temperature group respiration curves is in agreement with some of the results obtained by Agrell (1) for *Anagasta* (formerly *Ephestia*) *kühniella* and *Forficula auricularia*. Agrell did not establish a temperature–respiration curve over a range of temperatures in his acclimatized insects.

The constant b in the respiration curve reflects the degree of curvature of the T–R line over the total temperature range and represents the slope of the log–log line. The increase in b and the decrease in a in the 18° and 38° group curves compared with 30° controls is reflected in a counterclockwise "rotation" of the former about the region of maximum oxygen consumption, with respect to the curve for 30° controls. This situation is illustrated in the semilogarithmic curves for females in Fig. 2.

Rao and Bullock (27) have reported that the Q_{10} is lower in cold adapted poikilotherms than in those adapted to high temperatures, the constant varying with thermal adaptation. Scholander *et al.* (32), however, stated that the Q_{10} does not vary significantly with cold adaptation. Rao and Bullock themselves did not consider a change in Q_{10} in itself to be evidence for thermal adaptation. Bělehrádek (4) reported that the thermal coefficient rises in a number of cases with adaptation to a higher temperature. In the present work, the Q_{10} of the 18° adapted insects was greater at any given temperature than that of controls at the same temperature. The same also applies to the 38° insects.

Analysis has shown no significant difference in a or b between the 18° and 38° females. This apparent similarity may well be only superficial and is probably not due to similar metabolic states. The first evidence in favor of this is found in the mortality at extreme temperatures. It has been demonstrated that the survival of 38° insects at 40° C. was less than that of 18° insects at this temperature. Thus, long-term exposure to 38° C. did not enhance the ability of the insects to survive high temperatures. The 18° insects showed increased cold hardiness and slightly increased heat hardiness as well. Secondly, the 18° insects appeared, when stimulated, to be as vigorous as the 30° controls, or perhaps even more so. It was much more difficult to keep the former confined in a glass tray at room temperature, compared with 30° controls. The 38° insects, however, were very slow moving at all times. Some

exhibited poor motor coordination after some time at 38° C. Finally, no respiratory compensation was exhibited in the 18° insects. The position of the 38° curve can be interpreted in either of two possible ways. Either the insects exhibited respiratory compensation at the high temperature, or their metabolism was depressed by adverse effects of hyperthermy. It does not seem probable that this species would show respiratory compensation at high temperatures without also exhibiting it at low temperatures.

When the insects are first transferred to 18° C. no respiratory compensation is evidenced; a "passive" lowering of the respiration occurs. This corresponds to the lower portion of the control T–R curve. When transferred to 38° C., however, a respiratory increase followed by depression occurs in both sexes. No definite reason for the depression can be given at this time. The 38° respiration depression may possibly have been the result of, first, a positive accelerating effect on respiration due to the higher temperature, followed by a deleterious, retarding effect.

An alternative explanation for the increased respiration and subsequent depression involves the possible differential effect of the high temperature on different tissues. The slow, prolonged increase in respiration following the initial rise may itself indicate detrimental effects of heat on the enzymatic processes. When the point of maximum respiration had been reached a particular group of tissues more sensitive to high temperatures than the rest may have succumbed completely. This would reduce the general respiration per unit weight to a lower level. The upper lethal temperature range for this species is very close to 38° C. (23).

Since both the sex and the ratio of active to inactive weight seem to have influenced values obtained for respiration per unit weight in the present work, these factors should at least be considered in all work of this kind. In situations wherein there may exist differences in the ratio of active to inactive weight between two temperature groups these considerations are especially applicable. An example is seen in comparing the respiration per unit weight of some marine invertebrates from different environmental temperatures utilizing dissolved carbonate. The difference in temperature would result in a difference in the availability of dissolved carbonate, as a result of shifting in the carbon-equilibrium equation. Hence, oxygen consumption values based upon a unit of weight would tend to give higher values in organisms from colder water due merely to a *relatively* smaller amount of inactive tissue present in, for example, the exoskeleton. This shift in the oxygen consumption values would be in the same direction as that previously reported in respiratory compensation studies (7).

B. Weight Changes

The increased weight in 18° insects was the result of an increased water content, possibly as a result of a decreased saturation deficiency at 18°. These results do not agree with the conclusions of Menusan (21) that weight changes at lower temperatures at the same relative humidity in *T. confusum* were not due to differences in the water content of the insects.

Undoubtedly, the increased water content, together with a decreased dry weight in 18° insects served to reduce the values for oxygen consumption on a unit weight basis. On this basis alone, however, the 18° curve would be expected to be depressed more at higher temperatures and less at the lower ones. This is not the case. Apparently some other factor, in addition to artifact, seems to have been responsible for depression of the 18° curve as proposed by Agrell (1). This additional factor seems to have been reflected in the increased curvature (*b*) in the 18° curve.

The differential effect of weight on the respiration within a given temperature group would not have substantially influenced the T–R curves. The latter were determined on the basis of the mean respiration of five samples of insects at each temperature.

C. Survival at Extreme Temperatures

The sex difference in survival ability at −3° C. in 30° control insects was not indicated at 40° C. It is difficult to be certain as to whether this differential survival ability at the low temperature was due primarily to factors related directly to sex itself or to the factor responsible for the difference in weight between the two sexes. Lighter females were more sensitive to temperature change, while heavier males were the more sensitive. In other words, "lightness" in males may have more survival value and in females "heaviness" may have more survival value at the lower temperatures. A more direct factor may have been chiefly responsible for the mortality at −3° C. This factor was more critical in females than in males.

Long-term exposure of *T. confusum* to 18° increased heat resistance as well as cold resistance, as evidenced by a greater LD_{50} at 40° C. in the 18° insects. The acclimatizing temperature of 18° C. is far from the physiological optimum of the insects (14). The temperature factor in the environmental resistance should increase away from the physiological optimum. It is possible that an increase in environmental resistance in the form of a reduction of the temperature to 18° C. was counteracted by an increase in thermal resistance in the insect. This increased resistance, as well as enhancing the survival ability at low temperatures, may also have served in some manner to increase the resistance to high temperatures.

The greater water content in 18° insects, together with the increased survival ability at both −3° C. and +40° C. constitutes some evidence against theories concerning a positive relationship between desiccation and thermal resistance (2, 6, 25, 28). Nevertheless, the possibility of a positive relationship between protoplasmic viscosity and thermal resistance cannot be excluded. Heilbrunn (15) has shown that eggs of the sea urchin *Arbacia* in sea water diluted with distilled water exhibit increased protoplasmic viscosity through coagulation when the dilution is 60–70% distilled water. On the basis of arguments put forth by Bělehrádek (5) the increased coefficient *b* in the T–R curves of 18° insects seems to constitute evidence for increased protoplasmic viscosity.

The reduction in the viability of *T. confusum* exposed to 38° C. for a prolonged period may have resulted, at least partially, from excessive dehydration. The importance of the time factor in heat death has been emphasized by Orr (22). In the present work, 4.02 days at 40° C. has been shown to be the time necessary to kill 50% of control insects, at a relative humidity of 75%. Impairment, and possibly death, at 38° C. under the same conditions of humidity would mean extension of the time factor. The latter might be in the vicinity of 6 or 7 months, the time during which the present insects were subjected to 38° C.

D. The Temperature Coefficient

Most reports in the past have shown that Q_{10} decreases with rising temperature in many biological reactions (17). The present work has supported this. This variation in Q_{10} introduces the variable *temperature range* into comparisons of Q_{10} between organisms.

In cases where acclimatization results in horizontal shifting of the temperature–metabolism curve (7), comparisons on the basis of Q_{10} are particularly confusing. A given temperature range applies to different relative portions of the curve. For example, a given T–R curve may be shifted to the left when a poikilotherm exhibits respiratory adaptation to cold. At any given temperature the respiration of the cold adapted form is higher than that of the organism adapted to the higher temperature. Therefore, if a given temperature range along the x axis is projected upward through the T–R curves, it will cut off a section on the cold-adapted curve which is much nearer the latter's maximum thermal limit than the section cut off on the warmer-environment curve by the same projection. Hence, since the Q_{10} normally decreases upward along the T–R curve, one might tend to conclude that cold adaptation, in addition to resulting in increased respiration, shows a lower Q_{10} if only this particular temperature range is considered.

The critical thermal increment (μ) in the Arrhenius equation represents another source for confusion. Observation of the semilogarithmic plots *without* the lines drawn through them frequently gives more an impression of a continuous curve, rather than two or more straight lines. The exponential Arrhenius equation represents an assumption that the semilogarithmic line would be straight. Some excellent examples of this situation have been given by Bělehrádek (5).

Bělehrádek has stated that the straight line proposed originally by Krogh, and the Arrhenius equation, are only special cases of a more general situation described by the log–log type equation. The constant b in the present work, which might be referred to as the "thermal-sensitivity coefficient", might be useful in comparing T–R curves of the log–log type in various experimental groups. The constant takes into account the degree of response of the organism to temperature change over the total effective temperature range, and does not vary with the temperature range chosen.

Conclusions and Summary

1. The present work has shown no significant difference in the oxygen consumption per unit weight between male and female adult *T. confusum*. However, there is a trend in the direction of a higher respiration rate in females.

2. Prolonged exposure to 18° C. resulted in a lowering of the T–R curve, as did prolonged exposure to 38° C. In both cases the curves exhibited increased curvature and tended to approach that for control 30° insects near the upper thermal limit.

3. Acclimatization was evidenced in the 18° insects by increased survival ability at −3° C. and not by respiratory compensation. There was no evidence for acclimatization in the 38° insects.

4. In control 30° *T. confusum*, males survived exposure to −3° C. to a greater degree than females. This sex difference in survival at the low temperature was eliminated by prolonged exposure to 18° C. Lighter females and heavier males were more sensitive to temperature changes. There was a corresponding sex difference in the respiration–weight relationship.

5. Female *T. confusum* possessed a greater wet and dry weight than males. Changes in the amount of respiring tissue in proportion to the amount of water were probably reflected in depression of the T–R curve of 18° insects. Some additional depressing factor was probably also involved.

6. Both sex and the ratio of active to inactive weight in *T. confusum* may influence the respiration–weight regression and also the numerical value of the oxygen consumption per unit weight. These factors should be taken into account when dealing with any problem of this kind.

7. The temperature–respiration curves in the present work were adequately described by a double-logarithmic type equation within the limits of the experiments. The constant *b* in the equation, or "thermal-sensitivity coefficient", might be more valuable in comparing temperature–metabolism curves of various temperature groups than exponential constants based upon semi-logarithmic interpretations. The former is independent of the temperature range chosen.

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DESCRIPTIONS OF SIX NEW SPECIES OF GARMANIA NESBITT AND LASIOSEIUS BERLESE (ACARINA: ACEOSEJIDAE)[1]

D. A. CHANT[2]

Abstract

Six new species of aceosejid mites are described and figured: *Lasioseius* (*Lasioseius*) *allii* from onion bulbs imported from South Africa to New York; *L.* (*Paragarmania*) *helvetius* from pear bark, Lausanne, Switzerland; *L.* (*P.*) *bakeri* from dried prunes and peaches imported to California from Chile, and on cantaloupe fruit imported to Arizona from Mexico; *Garmania curtipilis* from sorghum imported to Texas from Mexico; *G. longipilis* from cativo logs imported to North Carolina from Costa Rica; and *G. utahensis* from decayed tomato fruits in Utah. A short diagnosis is given for each species.

Introduction

The genus *Garmania* Nesbitt was placed in the family Phytoseiidae Berlese (Nesbitt (3)), but in a recent revision Evans (2) transferred it to Aceosejidae Baker and Wharton, and moved the subgenus *Paragarmania* Nesbitt from *Garmania* to *Lasioseius*. These two genera are distinguished from most others of this family by having a pair of setae on the genital shield, by lacking lateral incisions on the dorsal shield, and by having a number of marginal setae on the interscutal membrane. *Garmania* is further separated by having only a simple anal plate that is remote from the genital shield, and *Lasioseius* is further separated by having a true ventrianal shield and normal chelicerae and by lacking jugularia.

Nesbitt (3) erected *Garmania* for six species that were originally described by Oudemans in the genus *Typhlodromus*. Nesbitt assigned four of these to the subgenus *Garmania*, because the adult females have simple anal plates without preanal setae, and the remaining two to the subgenus *Paragarmania*, because the females have a true ventrianal shield with a number of preanal setae on its anterior portion. By Evans' revision, all species that remain in *Garmania* have only an anal plate. *G. pomorum* (Oudemans) and *G. hypudaei* (= *bulbicola*) (Oudemans) were transferred to *Jordensia* by Evans because they have the marginal setae on the dorsal shield instead of on the interscutal membrane. In *Lasioseius*, *Paragarmania* is separated from *Lasioseius* sensu stricto by having only a few weak teeth on the fixed digit of the chelicera as compared with the multidentate condition of the latter.

The following are descriptions of six new species collected by the author or loaned by Dr. E. W. Baker.

Lasioseius (*Lasioseius*) *allii* sp. n.

(Figs. 1–3)

Female.—Length 442 μ; width 257 μ. Dorsal shield entire, with 34 pairs of simple setae (Fig. 1). Most setae on dorsal shield shorter than distances

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between their bases. Sternal shield with lateral extensions between coxae I and II that originate anterior to first pair of setae, and with only two pairs of setae; a third and fourth pair of setae on membrane posterior to shield. Metasternal plates absent. Genital shield narrow, truncate, with a pair of setae. Peritreme extending from epistome to coxa IV, its posterior end short, blunt, and not curving around coxa. One pair of metapodal plates. Ventrianal shield as long as wide (113 μ), rectangular, with two pairs of preanal setae (Fig. 2). Six pairs of setae on membrane surrounding ventrianal shield. Fixed digit of chelicera long, with seven denticules and pilus dentilis; movable digit with two small teeth (Fig. 3). Legs without macrosetae.

Male.—Like female, but ventrianal shield with five pairs of preanal setae. Chelicera with bulbous spermatophoral process. Setae on dorsal shield longer in proportion to body than in female.

Diagnosis.—The presence of only two pairs of preanal setae on the ventrianal shield of the adult female is distinctive of the species.

Locality and type material.—The holotype and a second female, collected at New York City on May 11, 1939, on onion bulbs imported from South Africa, are in the United States National Museum. Two females and two males, found at Brownsville, Texas, on September 6, 1947, on onions imported from Portugal, are at the Belleville laboratory.

Lasioseius (*Paragarmania*) *helvetius* sp. n.

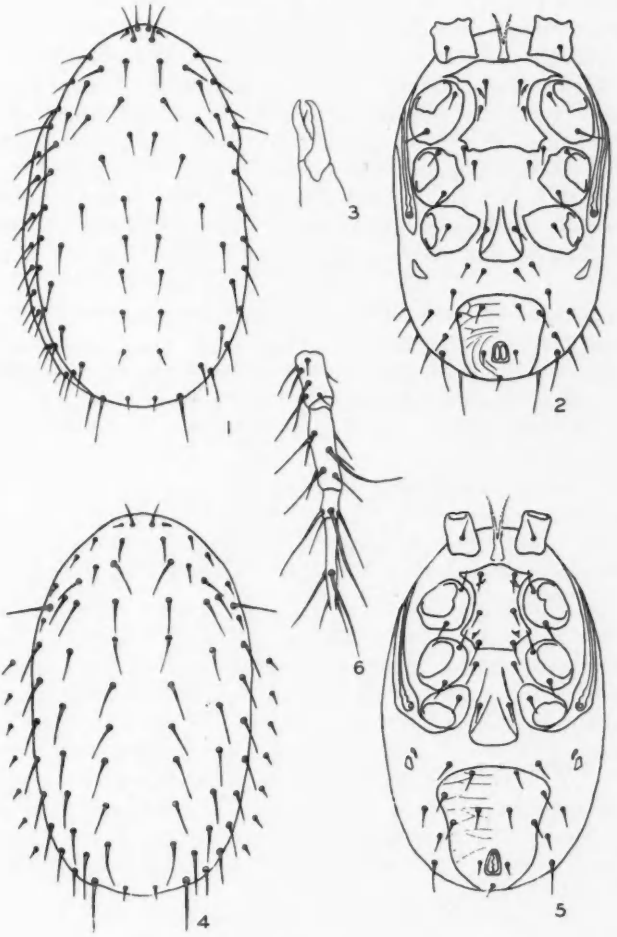
(Figs. 4–6)

Female.—Length 442 μ; width 275 μ. Dorsal shield entire, with 36 pairs of simple setae (Fig. 4). Some setae on anterior edge of shield very short; remaining setae as long as distances between their bases. Sternal shield with lateral extensions, and with three pairs of setae. Metasternal plates absent. Genital shield narrow, truncate, with a pair of setae. Peritreme of normal length with posterior portion curving slightly around base of coxa IV. Two pairs of metapodal plates. Ventrianal shield longer (140 μ) than wide (113 μ), rectangular, with lateral margins slightly concave, and with four pairs of preanal setae (Fig. 5). Four pairs of setae on membrane surrounding ventrianal shield. Chelicera with many teeth. Leg IV with many long setae on genu, tibia, basitarsus, and tarsus (Fig. 6).

Male.—Unknown.

Diagnosis.—The presence of four pairs of preanal setae on the ventrianal shield is distinctive, as well as the short setae on the anterior edge of the dorsal shield and the many long setae on leg IV.

Locality and type material.—The holotype, found at Lausanne, Switzerland, in October, 1953, on pear bark, is in the Canadian National Collection (No. 6642). A second female, with the same collection data, is at the Belleville laboratory. Both were collected by the author.



FIGS. 1–6. 1. *L.* (*L.*) *allii* sp.n., female dorsal surface. 2. Ventral surface. 3. Chelicera. 4. *L.* (*P.*) *helvetius* sp.n., female dorsal surface. 5. Ventral surface. 6. Leg IV.

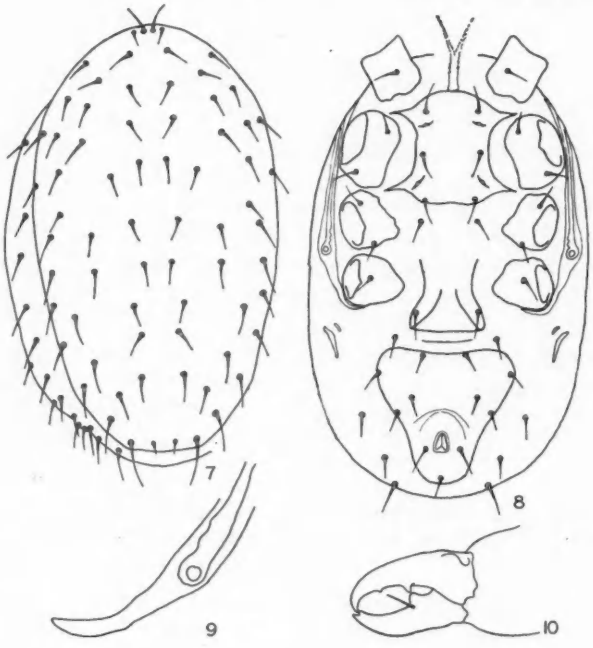
Lasioseius (*Paragarmania*) *bakeri* sp. n.

(Figs. 7–10)

Female.—Length 509 μ; width 320 μ. Dorsal shield entire, with 35 pairs of simple setae (Fig. 7). Setae on dorsal shield shorter than distances between their bases. Sternal shield with two lateral extensions, between coxae I and II, and II and III, and with three pairs of setae. Metasternal plates absent. Genital shield narrow, truncate, with one pair of setae. A lateral fold between genital and ventrianal shields. Peritreme of normal length and curving slightly around base of coxa IV (Fig. 9). Two pairs of metapodal plates. Ventrianal shield triangular with lateral margins concave, as wide as long (163 μ), with four pairs of preanal setae (Fig. 8). Four pairs of setae on membrane surrounding ventrianal shield. Fixed digit of chelicera with two teeth and pilus dentilis; movable digit with two teeth (Fig. 10). Legs without macrosetae.

Male.—Ventrianal shield with five pairs of preanal setae. Chelicera with short spermatophoral process.

Diagnosis.—This species most closely resembles *L.* (*P.*) *amboinensis* Oud. (5) and *L.* (*P.*) *mali* Oud. (6) but differs from the former in the length of the setae on the dorsal shield (longer in *amboinensis*), the number of metapodal



FIGS. 7–10. 7. *L.* (*P.*) *bakeri* sp.n., female dorsal surface. 8. Ventral surface. 9. Peritreme. 10. Chelicera.

plates, the shape of the sternal shield, the shape and dentition of the chelicerae, and the shape of the peritreme. It differs from *L.* (*P.*) *mali* in the length of the setae on the dorsal shield, the shape of the sternal shield, the number of setae on the membrane surrounding the ventrianal shield, the number of metapodal plates, and the shape of the peritreme.

Locality and type material.—The holotype, 17 other females, and three nymphs, found at Los Angeles, California, on August 1, 1949, and March 15, 1951, on dried prunes and peaches imported from Santiago, Chile, are in the United States National Museum. Two females, collected at Nogales, Arizona, on June 12, 1949, on cantaloupe fruit from Mexico, and seven females and one male collected at New York City on July 11, 1949, on lemons from Italy, are at the Belleville laboratory. The species is named in honor of Dr. E. W. Baker.

Garmania curtipilis sp. n.

(Figs. 11–14)

Female.—Length 446 μ; width 208 μ. Dorsal shield entire, with 34 pairs of simple setae (Fig. 11). Setae on dorsal shield much shorter than distances between their bases. Anterior of three pairs of setae on hypostome thickened and thornlike (Fig. 12). Sternal shield normal. Metasternal plates absent. Genital shield normal. Peritreme of normal length with a subsidiary, posterior plate (the exopodal plate) extending from stigma around base of coxa IV (Fig. 13). Two pairs of metapodal plates. Anal plate small, oval, longer (81 μ) than wide (68 μ), and without preanal setae (Fig. 14). Nine pairs of setae on membrane surrounding anal plate. Legs without macrosetae.

Male.—Unknown.

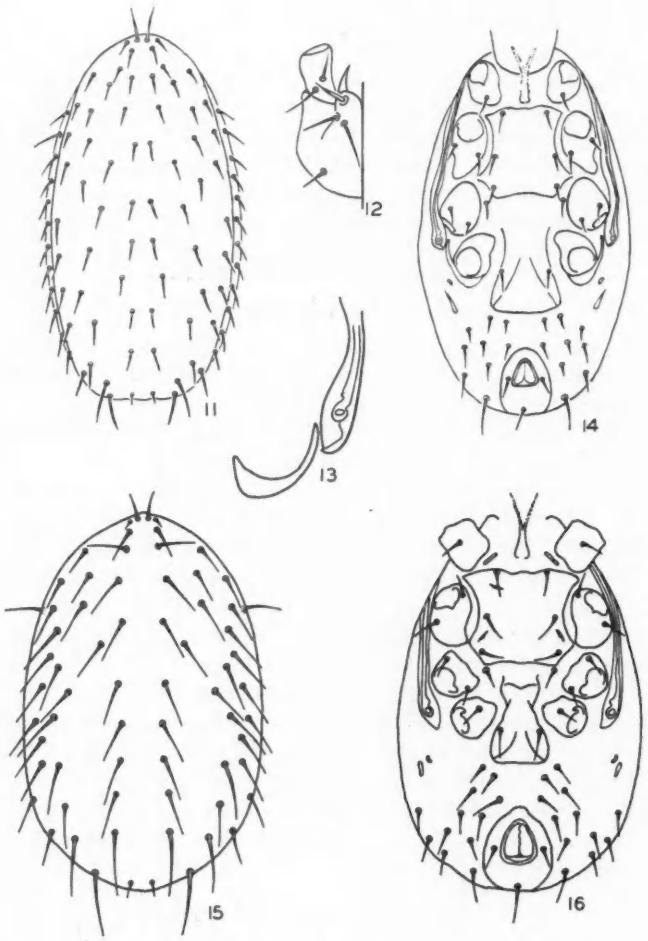
Diagnosis.—The greatly thickened pair of setae on the hypostome distinguishes this from all other known species except *Jordensia hypudaei* (Oudemans (4)). This species differs from *hypudaei* in the length of the setae on the dorsal shield (shorter), the shape of the peritreme, and the chaetotaxy of the posterior ventral membranous region, and by having the marginal setae on the interscutal membrane instead of the dorsal shield.

Locality and type material.—Two females and a nymph, found on sorghum from Mexico at Brownsville, Texas, on November 23, 1935, are in the United States National Museum.

Garmania longipilis sp. n.

(Figs. 15, 16)

Female.—Length 433 μ; width 244 μ. Dorsal shield entire, with 33 pairs of setae (Fig. 15). Setae on dorsal shield much longer than distances between their bases. Setae on hypostome normal. Sternal shield with lateral extensions, and with three pairs of setae. Metasternal plates present, each with a seta. Genital shield normal. Peritreme of normal length anteriorly but not extending behind coxa IV. Two pairs of metapodal plates. Anal plate small, oval, longer (104 μ) than wide (86 μ), without preanal setae and with anterior



FIGS. 11–16. 11. *G. curtipilis* sp.n., female dorsal surface. 12. Hypostome. 13. Peritreme. 14. Ventral surface. 15. *G. longipilis* sp.n., female dorsal surface. 16. Ventral surface.

margin bulbous and slightly pointed (Fig. 16). Nine pairs of setae on membrane surrounding anal plate. Both digits of chelicera with many denticules. Leg IV with one macroseta.

Male.—Unknown.

Diagnosis.—The shape of the anal plate distinguishes this from all other known species of the genus except *G. bickleyi* Bram (1). It is distinguished from *bickleyi* by the much longer setae on the dorsal shield, by the presence of metasternal plates, and by the presence of the macroseta on leg IV.

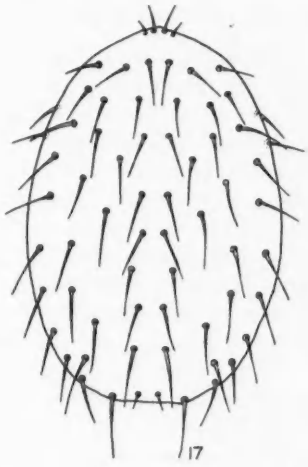
Locality and type material.—Seven females, collected at Charleston, North Carolina, on February 5, 1951, on cativo logs from Costa Rica, are in the United States National Museum.

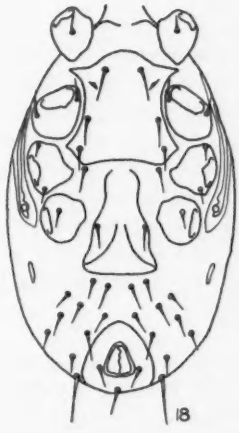
Garmania utahensis sp. n.

(Figs. 17, 18)

Female.—Length 464 μ; width 275 μ. Dorsal shield entire, with 32 pairs of simple setae (Fig. 17). Setae on dorsal shield much longer than distances between their bases. Setae on hypostome normal. Sternal shield with short lateral extensions, and with three pairs of setae. Metasternal plates absent. Genital shield normal. Peritreme not extending beyond coxa IV. One pair of metapodal plates. Anal plate small, oval, longer (90 μ) than wide (77 μ), with anterior margin rounded and without preanal setae (Fig. 18). Ten pairs of setae on membrane surrounding anal plate. Chelicera with many teeth. Legs without macrosetae.

Male.—Ventrianal shield with seven pairs of preanal setae. Chelicera with spermatophoral process.





FIGS. 17–18. 17. *G. utahensis* sp.n., female dorsal surface. 18. Ventral surface.

Diagnosis.—Most closely resembling *G. domestica* Oud. (6) but distinguished from that species by the longer setae on the dorsal shield and by the shape of the sternal shield.

Locality and type material.—The holotype, three other females, and three males, collected at Farmington, Utah, on November 13, 1952, on decayed tomato fruits, are in the United States National Museum.

Acknowledgments

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ON THE POPULATION REDUCTION OF CHORIOPTIC MANGE MITES ON CATTLE IN SUMMER[1]

GORDON K. SWEATMAN[2]

Abstract

Extrinsic factors appear to be significant in reducing the number of chorioptic mange mites on pastured cattle in summer, and an environmental "change" per se and intraspecific factors may be limiting factors also. Molting, normal desquamation, rubbing, licking, and any physiological skin change are *not* deleterious factors to a population of *C. bovis*.

Introduction

Chorioptes bovis (Hering, 1845) Gervais and van Beneden, 1859 is a species of mange mite found throughout the world on most classes of domestic livestock as well as on some zoo animals. Sweatman (5, 6) has described the biology of this parasite and nature of its infestations. Sometimes the mite is associated with a mild dermatitis referred to colloquially as "barn itch" when on cattle or "foot mange" when on horses. It is well documented by writers in temperate climates that the dermatitis and the mites become greatly reduced on, and sometimes virtually eliminated from, the hosts when they are on pasture during summer. However, in the current studies a large general infestation of *C. bovis* was maintained experimentally on cattle in summer by keeping them indoors in stanchions (5). This indicated that reduction in the number of mites was related to summer pasture conditions. The current study was designed to determine the reason(s) for this phenomenon. A number of theoretical possibilities may be conjured up as explanations, some of which are discussed below under five subheadings.

Experiments on the Population Reduction

(*a*) *Molting and Normal Desquamation*

The effect of hair-molting and desquamation which could mechanically carry off mites will be dealt with rationally rather than experimentally. Duerden and Whitnall (1) observed that hair-shedding by cattle in South Africa is in progress throughout the year. In Canada, molting in springtime is usually an inconspicuous process on dairy cattle that have been in barns during winter, and the hair seldom mats or falls out in irregular patches ("cotting") as it does on animals like the camel and musk ox. It would seem that molting and desquamation are continuous and fairly constant processes on livestock, and it is being assumed that as many mites would be lost by this means in winter as in summer.

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Contribution from Animal Pathology Laboratories, Health of Animals Division, Canada Department of Agriculture, in co-operation with the Institute of Parasitology, Macdonald College, Macdonald College P.O., Que., Canada.
[2]Animal Pathology Laboratories, Health of Animals Division, Canada Department of Agriculture, Montreal Area Branch Laboratory, Macdonald College.

(*b*) *Auto-grooming*

Rubbing and licking by cattle in stanchions in winter are restricted essentially to areas on their front quarters. Animals with general mite infestations were maintained in non-rotating stanchions in the present experiments, and examinations for mites were made from the licked shoulder areas and from the non-licked areas contiguous with them. On three animals, both regions were infested and there were fewer mites in the licked areas, but probably no fewer than would be expected on a more anterior part of the body (see Ref. 5). The animals also licked their front feet, and these were often heavily infested. Snowball (4) observed that licking contributes to the reduction of the numbers of the tick *Boophilus microplus* on cattle, but these are much larger than *C. bovis* and are probably affected directly by the rasping of the tongue. The results above indicate that auto-grooming by animals in stanchions is not excessively, if at all, detrimental to a mite population.

This same problem was also approached in summer. It is noteworthy that vigorous rubbing and licking generally occur when animals, whether infested or not, are on pasture for the first time in spring, and it is soon after this that there is a noticeable drop in the size of the mite population. In the current experiments, two cows with general mite infestations were maintained

TABLE I

PERIOD OF DISAPPEARANCE OF MITES FROM DIFFERENT SITES ON CATTLE AND THE SUBSEQUENT SUITABILITY OF EPIDERMAL MATERIAL FROM THOSE SITES FOR REARING MITES IN VITRO

Animal number	Surface area	Period for disappearance of mites, days	Subsequent period for in vitro life cycle, days
1 (isolated)	Middle back	7	21
	Base of tail	7	21;26
	Buttocks	14	21
	Hind foot	Positive throughout	20
2 (isolated)	Middle back	13+	–
	Base of tail	13	21
	Buttocks	76	–
	Feet	76	19
3 (in herd)	Base of tail	7	22
	Buttocks	98	24
4	Buttocks	62	23
5	Base of tail	31	–
	Buttocks	31	23
6	Base of tail	7	26
7	Base of tail	45	24
	Buttocks	37	–
8	Buttocks	7	25
9	Buttocks	40	–
10	Buttocks	Positive throughout	23

individually on pastures surrounded by electric fences. Each animal was allowed complete freedom within its pasture, but lateral movements of the head were prevented by means of a slat-board collar. This prevented licking; the electric fence rubbing. Cattle do not roll. Vertical head movement was not inhibited by the collar so that the beast could feed normally. One animal (No. 1 in Table I) had been on pasture for only 7 days (June 7 to 14, 1956) when it was no longer possible to find mites on its torso, including the base of the tail. Mites persisted, however, on the lower hind legs. After 2 weeks (June 6 to 19, 1956) on pasture, no mites were found on the torso or base of the tail of the second animal (No. 2 in Table I) with the exception of a single mite located in the middle of the back on the 33rd day. Mites persisted from the escutcheon to the feet of this animal from June 6 to August 21, but in chronologically decreasing intensity. After August 21, no mites were ever located again on this individual. (Lesions that had persisted at the hocks since the animal was placed on pasture disappeared by August 30.) On these two animals, the mites disappeared despite the fact that the animals could not auto-groom. Auto-grooming is not necessary, therefore, for a reduction in the number of mites.

(*c*) *Physiological Skin Changes*

Most cattle have a fairly sudden change in the kind and sometimes quality of feed when first placed on pasture in spring. This, presumably, could affect the constitution of the sloughed epidermis on which the mites feed to such an extent as to be detrimental to these minute parasites. Sweating must be considered also. The amount of water secreted by the skin of cattle varies according to the conditions under which the beasts are maintained. In well-ventilated barns, and on pasture, water secreted by the skin is usually evaporated off at the same rate as it is produced with no visible accumulation of sweat. Therefore, physical drowning of the mites by this medium is insignificant. However, the sweat glands of domestic animals are apocrine, and the organic matter in their sweat might vary from season to season in such a way as to be lethal to *C. bovis*. The significance of both the above physiological processes was approached by collecting epidermic debris, hair, and the biochemical products of sweat in the following experiments.

Eight cows in a Holstein–Friesian herd had mites on their feet, base of the tail, and/or buttocks prior to being placed on pasture in spring. Other areas were negative or possessed too few mites to be detected. The herd was maintained on a typical eastern Canadian pasture with available shade trees, and the animals were not obliged to remain in direct sunlight as were the two previous animals corralled individually within electric fences. Besides the extrinsic factors, mites on these cattle were exposed to possible intraspecific factors and auto-grooming. After the animals were permanently on pasture (except for the daily milking routine), collections of material from the tail-base and buttocks were examined individually for mites every few days. When an area was first found negative, epidermal material was collected from that area and placed in a vial with about 200 eggs of *C. bovis*. The eggs were gotten

from culture vials of the mite, the technique for which was described elsewhere (6). The vial was then held under the in vitro conditions of 35° C., 80% R.H., and complete darkness suitable for culturing *C. bovis*, and observations followed on the development of the mites emergent from the eggs. Table I shows that the period necessary for the disappearance of mites on seven of the animals (Nos. 3 to 9 inclusive) varied from 7 to 45 days for those mites on the tail-base and from 7 to 98 days for those on the buttocks. In all cases tested, in vitro culture of the mites on the epidermal material showed that the life cycle was completed in the usual 3 week period established previously for naturally infested epidermal material collected in winter (6). This period was also identical for mites on epidermic debris from the two control areas (on animals Nos. 1 and 10 in Table I) that remained positive throughout the summer. It seems likely, therefore, that any physiological change that may have taken place in the skin or its secretions, hair, or epidermic debris was not deleterious to *C. bovis*.

(*d*) *Intraspecific Factors*

Chorioptic mites are so gregarious that myriads of them will occur on a small area on a host. Their habits, like egg-laying, are frequently communal in nature (6). At times, however, mites for no obvious reason begin to disappear from the host when it is still stabled. Sweatman (5) noted that the incidence of infestation in a herd began to drop in late winter some eight weeks before the animals were pastured. A frequent explanation conjured up when other parasitic organisms do likewise is that acquired resistance has developed by the host. Before pursuing this further, it is desirable to present observations made on one animal that had been maintained continuously indoors for 20 months, during which time it had developed a large general infestation. After this period, the animal was transferred to a different barn and maintained by itself. The mites then began to disappear rather suddenly from the cow's body even though still indoors. At the end of 6 weeks no mites could be found anywhere on the animal. Was there a lethal factor coincidental with, or related directly to, the change of environment? Epidermal material was collected from various sites on the cow and set up in vials with eggs of *C. bovis* under suitable in vitro conditions for culturing. In all instances, the mites completed their life cycle in the usual 3 week period. Because of this, any acquired resistance by the host seemed unlikely, and it is untenable to suggest intraspecific competition for nutritive requirements. The rapid die-off appeared to be related to the mites themselves, and one cannot help wonder about the possibility of an epizootic. This would have been possible insofar as the individual cow is concerned, but would hardly explain the decrease in the numbers of mites observed (5) in a herd of stanchioned cattle in early spring.

(*e*) *Extrinsic Factors*

The first extrinsic factor to be discussed relative to the disappearance of mites on cattle on pasture will be rain and water. Some relevant experiments

were carried out under in vitro conditions for culturing *C. bovis* at 35° C. and 80% R.H. A total of 251 eggs in four different trials were placed in vitro on filter paper made wet with water. Five eggs failed to hatch, whereas larvae emerged from all control eggs that had been held under similar conditions on dry filter paper. Two exposures of water 24 hours apart under in vitro conditions apparently prevented a larger percentage of eggs from hatching. In one lot, 54 of 200 eggs failed to hatch, and in another 120 of 207 eggs did not hatch. Observations on the host using an isolation unit described by Sweatman and Pullin (7) showed that a single or double application of water prevented some eggs from hatching (27 of 169 eggs and 12 of 149 eggs that had been exposed once, and 10 of 239 eggs and 14 of 235 eggs that had been exposed twice), but not nearly the numbers observed in vitro. Water alone, therefore, that is in an environment suitable for its evaporation, prevents some eggs from hatching. Additionally, water was sprayed on a total of 122 eggs in four different trials that were kept continuously wet by changing the in vitro physical environment from 80% to 100% R.H. and 35° C. None of these eggs hatched. Continuous soaking is therefore lethal. Water, as it affects quiescent mites, was observed also. Most, but not all, survived one exposure of water under in vitro conditions. Two exposures of water 20 hours apart, however, killed all but 3 of 61 quiescent mites in three in vitro trials.

The effect of water on natural infestations on the host was determined (*a*) following a rainstorm, and (*b*) on members of a positive herd that walked across a river with water up to the dewlap four times a day to and from milking. In the first instance, the number of mites seemed to be about the same both before and after the storm. The cattle that crossed the river each day maintained mites on their feet for about as long as cattle in other herds on dry ground. The infestation within the herd persisted from one year to the next. Because of these in vitro and in vivo observations, one cannot ignore the effects of rain or water, but extensive permanent damage to the mite population by these factors seems unlikely, except perhaps where its effect is continuous such as might occur in tropical rain forests, or on animals that wallow in mud and water. Indian water buffalo do this in the rice paddies of Asia. This behavior would probably prevent this mammal from maintaining an infestation even though at least some individuals in the species appear to be physiologically suitable hosts (6).

Some effects of temperature were examined. As a preface to these observations, it is noteworthy that Galuzo (2) observed that the tick *Dermacentor* sp. on sheep in Russia infested the back of the host at night, and migrated to the abdomen and thorax during the day in the heat of the sun. He stated also that larvae of the tick *Hyalomma detritum*, which infested cattle in October and November, attached themselves to that part of the body most exposed to the sun, while the adults, which occurred in June and July, attached themselves to the underparts.

For the current experiment, the two cows maintained within the electric fences were utilized, together with four other cows in a herd. By the use of a

calibrated contact pyrometer, skin temperature determinations were made in the noonday sun (1 p.m., E.S.T.) on the warmest days of the 1956 summer near Montreal. It was a comparatively cool summer, and the air temperatures on these days ranged between 75° and 90° F., but were usually 80° to 85° F. The atmospheric relative humidities ranged between 41 and 61% at 1 p.m., E.S.T., on the days that temperatures were recorded. Since these were bright sunny days, the relative humidities at other times of the day were usually higher than those at 1 p.m. The recorded mean monthly relative humidities at 1 p.m. at nearby Dorval Airport were 64%, 60%, and 56% for June, July, and August respectively. Wind speeds, that might have an effect on the rate of evaporation from the skin and a consequent cooling effect, were generally around 11 m.p.h. The four animals in the herd, which had shade available, had surface (shoulder, middle-back, tail-base, and escutcheon) temperatures of 36° to 38.5° C. for two of them and 35° to 39.5° C. for the other two. Areas with white hair were generally about 0.5 C. degree cooler than areas of black hair. These temperatures were higher than the 31° to 35° C. noted for these same areas when the animals were stanchioned in barns in winter. The two animals within the electric fences that were exposed to direct sunlight had skin temperatures on the torso that ranged from 36° to as high as 43.5° C. (No sunburn occurred.) The temperature of their feet never reached that maximum temperature, but ranged between 36° and 41.5° C. Some cooling effect of the feet would be probable in pastures with long grass, particularly on damp ground. These torso surface temperatures were similar to those listed by Galuzo (2) for cattle on pasture in Tadzihikistan and eastern Uzbekistan, U.S.S.R. When the atmospheric temperature was 74° F., Galuzo observed that an animal in direct sunlight had a surface temperature of 43° C., whereas protection from the sun reduced the temperature to 36°C.

The skin temperatures observed in the current study were corroborated with some in vitro effects of these temperatures on the mites under darkness at a few relative humidities. Conditions* maintained at a constant 43.5° C.—the highest skin temperature observed—with a relative humidity of 80% and complete darkness killed many mites within a few hours and caused all to be moribund within eight hours. All were dead within 15 hours. A constant environment of 40° C., complete darkness, and 60% R.H. or 80% R.H. caused many mites to die in most culture vials within the first day, but some mites lived for 3 or 4 days. Although the environment of the mites on cattle would be more variable, these results suggest that the high skin temperatures experienced in the middle of the day in summer would be lethal to some, but not all, mites, and the temperature–humidity factor would be of some importance in the population reduction of *C. bovis* in summer.

*For these high temperature experiments it was necessary to replace the paraffin wax as shown in the previous report (6) with stainless steel plates elevated by legs and punched with holes of an appropriate size to support the glass vials. The sulphuric acid was replaced also with appropriate concentrations of potassium hydroxide or saturated salt solutions according to the data listed by Peterson (3).

From the foregoing discussion it seems possible to rule out molting, desquamation, auto-grooming, and physiological skin changes as deleterious factors to a population of *C. bovis*. The value of an environmental "change" per se or intraspecific factors may be important. When cattle are on pasture, extrinsic factors by themselves would appear to kill many *C. bovis*.

Acknowledgments

The co-operation and suggestions of Dr. J. W. Pullin, Officer-in-Charge of the Montreal Area Branch Laboratory of the Health of Animals Division, are much appreciated. Assistance in the field and laboratory was provided by Messrs. D. V. Conlon, J. Mackie, and W. Goulet.

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SOME NOTES ON A COLLECTION OF POLYCHAETA FROM THE NORTHEAST PACIFIC SOUTH OF LATITUDE 32° N.[1]

E. Berkeley and C. Berkeley

Abstract

Twenty-nine species of Polychaeta collected on, or near, the west coast of Lower California and of Mexico are recorded. Seventeen of them are pelagic. Of these, 10 are heteronereid phases of various Nereidae, in two of which cases (*Perinereis monterea* and *Nercis oligohalina*) neither of the heteronereid phases has been described previously. One species recorded (*Maupasia caeca*) is new to the northeast Pacific.

Introduction

The polychaeta with which these notes deal were collected by Mr. W. L. Klawe of the Inter-American Tuna Commission in 1955, 1956, and 1957 and submitted to us for identification. Many of them come from the west coast of Lower California and, of these, several have already been reported from the region (18), but a large number of the earlier records are from within the Gulf of California. Some special interest attaches to this collection because of the large proportion of heteronereids and other pelagic forms comprised in it. These resulted largely from Mr. Klawe's practice of collecting at night under a light whenever opportunity offered. In the notes which follow, references are given to descriptions of each species from which the synonymy and further references may be obtained.

Amphinomidae

Chloeia viridis Schmarda. Monro (23)

Two specimens taken under a light at Los Frailes, Mexico. Both are about 50 mm. long and 12 mm. wide as preserved. They agree with Monro's description except that the dorsal markings are indistinct. Differentiation from *C. pinnata* Moore and *C. entypa* Chamberlin, the other two species recorded from the northeastern Pacific, is based on the absence of any trace of a purplish or dusky spot associated with the lateral tentacles (23). No spurred setae could be found. Serrations on the notosetae in the median region of the body are, in most cases, quite heavy.

We have previously recorded the present species, and *C. pinata* Moore, from Lower California (1), differentiating them as above, but we still entertain the doubt as to the validity of the separation which we then expressed. It has been extensively recorded from the region by Hartman (18). In our 1939 paper we recorded its capture at a night light off Galapagos Island, and Hartman (18) mentions a similar instance at Agua Verde Bay, Gulf of California. It is interesting to note again this response to a light stimulus, particularly as there was no evidence of its association with sexual maturity.

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Contribution from the Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.

Glyceridae

Hemipodus californiensis Hartman. Hartman (17)

A single specimen, collected on a sandy beach at Ensenada, Lower California, agrees completely with the original description. This was based on material collected on the coast of California. The only subsequent record until the present one seems to be that of Rioja (31) from Mazatalan, Mexico.

Alciopidae

Callizona angelini (Kinberg). Fauvel (12)

Four pelagic specimens, taken, respectively, at the following stations: (1) 10° 03′ N. lat., 88° 52′ W. long., (2) 6° 55′ N. lat., 83° 38′ W. long., (3) 4° 16′ N. lat., 83° 56′ W. long., (4) 3° 41′ N. lat., 83° 00′ W. long., are typical of the species.

The species does not appear to have been recorded previously, as such, from localities so near to the equator. *Rhynchonerella pycnocera* Chamberlin was, however, taken at several stations in the vicinity by the Carnegie Expedition of 1928–1929 (39) and the characters of that species (6) are close enough to those of *Callizona angelini* to suggest their identity, as Monro (22) has already done.

Chamberlin (6) gives grounds for considering that the generic name *Rhynchonerella* should be substituted for *Callizona*, but we are inclined to agree with Monro (22) in thinking that the adoption of this synonymy "would only lead to confusion".

Greefia oahuensis McIntosh. Wesenberg-Lund (41)

A specimen taken in surface plankton at 9° 03′ N. lat., 104 °34′ W. long., is incomplete posteriorly. It measures 35 mm. for 55 segments and is 7 mm. wide. The ventral pedal glands are colorless, as described by Monro (22, 25). The species was originally recorded from Honolulu. This is the first subsequent Pacific record.

Corynocephalus paumotanus Chamberlin. Chamberlin (6)

Four specimens in plankton at 10° 52′ N. lat., 88° 02′ W. long., average about 10 mm. in length and 2 mm. in width. The species was taken by the Carnegie Expedition of 1928–29 in the same neighborhood (39).

Phyllodocidae

Lopadorhynchus (subgen. *Lopadorhynchus* s. str.) *uncinatus* Fauvel. Fauvel (12)

A single specimen taken in a plankton tow from 450 m. at 20° 00′ N. lat., 110° 35′ W. long. The specimen is 16 mm. long, about 3.5 mm. wide, and consists of 30 segments. It agrees with Fauvel's description in all respects except that the termination of the ventral cirri in parapodia posterior to the third is much more extended than described and illustrated (12, Fig. 67*e*) resembling in this respect the condition found in *Lopadorhynchus* (subgen. *Prolopadorhynchus*) *appendiculatus* (35). No pigment remains in the eyes.

Dales' record of the species from Monterey Bay, California (8), was the first from the Pacific. The present one extends the distribution considerably further south; Treadwell (39) records *L. nans* Chamberlin and *L. varius* Treadwell from approximately the same latitude as the present specimen. These are both distinguishable from *L. uncinatus* by the presence of simple setae in some parapodia other than the first three. Moreover, *L. nans* has three anterior segments with exclusively simple setae, whilst *L. uncinatus* has only two. The former species is regarded by Wesenberg-Lund (41) as synonymous with *L. brevis* Grube.

Lopadorhynchus (subgen. *Lopadorhynchus*) *brevis* Grube. Wesenberg-Lund (41, p. 12)

Two specimens taken in surface plankton at 3° 03′ N. lat., 101° 35′ W. long., and at 10° 52′ N. lat., 88° 02′ W. long., respectively, agree with Wesenberg-Lund's description. One is 12 mm. long and 4 mm. wide and consists of 25 segments, the other somewhat smaller. The species is regarded by Wesenberg-Lund and by Monro (22, p. 78) as synonymous with *L. nans* Chamberlin which has been recorded by Treadwell (39, p. 33, and Map 1, p. 56) from approximately the same latitude and both east and west of the present record.

Maupasia caeca Viguier. Fauvel (12, p. 190)

In surface plankton at 1° 58′ N. lat., 83° 49′ W. long. A single specimen 6 mm. long, about 1.5 mm. wide (without setae), and consisting of 17 setigers. The only previous record of the species in the Pacific is that of Ushakov (40, p. 268) from 43° 08′ N. lat., 156° 08′ E. long.

Nereidae

All the Nereidae in the collection except *Nereis* (*Neanthes*) *succinea*, were taken in the heteronereid phase (see Introduction). In many cases the identification of species in this phase is difficult owing to the modification of the posterior parapodia for swimming and has to be based largely on the paragnaths and the number of anterior setigers without swimming setae. In the key to the species occurring in the collection these characters are used as far as possible. Most of the species recorded were males. Figures given in boldface at the end of each entry in the key indicate the number of setigers anterior to the first epitokous foot in the male.

Key to Species

1. Paragnaths absent; three-regional body; 16–34 ..... *Nicon mexicana*
   Paragnaths present **2**
2. All paragnaths conical **3**
   Some paragnaths bars **8**
3. All paragnaths present **4**
   Group V of paragnaths absent **5**
4. Anterior notopodia with three lobes; 15–16 ..... *Nereis* (*Neanthes*) *succinea*
   Anterior notopodia with two lobes. 16–17 ..... *Nereis* (*Neanthes*) *oligohalina*
5. Paragnaths of Group VI, four or five large cones **6**
   Paragnaths of Group VI, small points **7**

6. Paragnaths of Groups VII and VIII, several irregular rows with the largest ones in the anterior row; 14–20 ..... *Nereis (Nereis) pelagica*
   Paragnaths of Groups VII and VIII, several irregular rows, all of nearly equal size excepting some smaller ones on the anterior part of VII; 14 ..... *Nereis (Nereis) grubei*
7. Paragnaths of Group VI, five to nine in two rows; 14 ..... *Nereis (Nereis) veleronis*
   Paragnaths of Group VI, six to ten in oval groups; 15–30 ..... *Nereis (Nereis) zonata*
8. Paragnaths of VI, smooth bars; 22 ..... *Perinereis monterea*
   Paragnaths of VI, pectinated **9**
9. Paragnaths of Groups I, II, and V absent **10**
   Paragnaths of Groups I, II, and V present; 14 ..... *Pseudonereis gallapagensis*
10. Simple heavy falcigers in some anterior parapodia; 16–25 ..... *Platynereis dumerilii* var. *agassizi*
    No simple heavy falcigers; 14 ..... *Platynereis polyscalma*

Nicon mexicana (Treadwell). Treadwell (38, p. 1 as *Leptonereis*), Hartman (20)

Two specimens taken at a night light off shore at Marina Madre Island, Mexico. Both are three-regional male heteronereids and appear to be identical; one is, however, so small that examination is difficult. The other is 25 mm. long and agrees well with the descriptions of the species except that there are fewer anterior setigers without swimming setae than is indicated (40 instead of "at least 55"). Hartman (20) altered Treadwell's generic designation from *Leptonereis* to *Nicon* on the ground that the unchanged posterior parapodia have no foliaceous dorsal lobes. Claparède (7) and Fauvel (12) include *Nicon* in *Leptonereis*.

The original, and only, previous records of this species are from stations in the Gulf of California, in the general neighborhood, but considerably farther north, than the present one.

Nereis (*Neanthes*) *succinea* (Leuckhart). Fauvel (12)

Two specimens, both in atokous phase, found on driftwood. The first, taken off Colombia at 6° 35′ N. lat., 78° 03′ W. long., is 22 mm. long, the second taken off Rio Anton, Gulf of Panama, is about 10 mm. long. Both agree with descriptions except that no paragnath can be detected for Group V on the smaller one. The species was recorded from the coast of Guatemala and that of Costa Rica by Ehlers (10, p. 118, as *N. acutifolia*), from the Panama region by Monro (23), and from the Gulf of California by Rioja (33).

Nereis (*Neanthes*) *oligohalina* Rioja. Rioja (32)

Four male heteronereids taken at a light at Hipolito Bay, Lower California, varying in length from 12 mm. to 15 mm., correspond closely with Rioja's description, so far as agreement can be determined in the heteronereid phase. The characters of the prostomium and those of the anterior parapodia posterior to the seventh agree with his figures (32, Figs. 13 and 15). The arrangement of the paragnaths is in accordance with his description as amended (34). There are 17 anterior setigers without swimming setae. The anterior 12 to 14 setigers have irregular dark spots which, jointly, form three or more longitudinal lines. Rioja's records are from brackish waters in the Gulf of Mexico.

Nereis (*Nereis*) *pelagica* Linné. Fauvel (12)

Four male heteronereids taken at a light in Hippolito Bay, Lower California, two about 20 mm. long, the others about 14 mm., agree with this species

except that there are only 14 anterior setigers without swimming setae instead of the more usual 16. A cosmopolitan species.

Nereis (*Nereis*) *grubei* Kinberg. Reish (30)

A single male heteronereid from Ensenada, Lower California, is typical. The species has been recorded under a variety of synonyms from California (30). It has also been recorded from the Gulf of California and from the west coast of Mexico (as *N. pseudoneanthes* Hartman) by Rioja (34).

Nereis (*Nereis*) *veleronis* Hartman. Hartman (18)

Three male heteronereids, taken at lights. Two specimens, from Abreojo Point, Lower California, are about 20 mm. long, the third, from Guayquil Bank, Ecuador, is a little larger. All agree with Hartman's description. The homogomph notopodial falcigers which are present in the atokous phase of the species (18, Fig. 73) do not appear until about the 30th setiger and are excluded from the epitoke. The species is recorded previously from Peru.

? *Nereis* (*Nereis*) *zonata* Malmgren var. *persica* Fauvel. Fauvel (11)

Six male heteronereids ranging in length from 3.5 mm. to 1.5 mm. taken at Hipolito Bay, Lower California, at a light, agree in respect of the paragnaths, the shape of the anterior setigers without swimming setae, and the form of the specialized dorsal homogomph falciger with the atokous phase of *N. zonata*. This falciger occurs in the present specimens as far forward as the 30th setiger, where the change to the heteronereid foot has already begun. The only character which disagrees with descriptions of the epitoke of *N. zonata* is the position in the body at which the change to the epitokous foot occurs. Instead of only 15 setigers without swimming setae in the anterior region, the specimens have 30 or more.

N. zonata is characteristically a northern species, but Fauvel (14) describes the variety *persica* from the Persian Gulf, which he says (11) scarcely differs from the type. We refer the present specimens as above with some hesitation.

Perinereis monterea (Chamberlin). Chamberlin (5)

Five specimens of the female heteronereid from Los Frailes, Mexico, ranging in length from 25 to 50 mm., and one of the male from Cedros Island, South Bay, Lower California, 40 mm. long; all taken at night lights. Neither of the heteronereid phases of the species has been recorded previously. There are 23 anterior setigers without swimming setae in the female, 22 in the male. The atoke was originally reported from Monterey Bay, California, and since from several Californian stations. It is recorded from Mexico by Rioja (31).

Pseudonereis gallapagensis Kinberg. Gravier (16), Hartman (18)

A single specimen of the male heteronereid, 10 mm. long, taken at a light at Sihuatanejo, Mexico. The species is widely known from the region covered in this paper.

Platynereis dumerilii Audouin and Milne-Edwards var. *agassizi*. Berkeley and Berkeley (3)

Six male heteronereids taken at lights. One at Hipolito Bay, Lower California, the remainder at Cedros Island, Lower California. This variety occurs widely in the northeast Pacific north of the region dealt with in this paper. Most of the previous records are of atokes, but the epitokes have been described. Rioja (34) records the atokous form from the south coast of Lower California and from stations in the Gulf of California.

Platynereis polyscalma Chamberlin. Chamberlin (6), Hartman (18)

Three male heteronereids about 25 mm. long taken at a light at Los Frailes, Mexico. Chamberlin described only the epitokes and these have been taken more recently in widely separated regions. The present specimens agree with Chamberlin's description of the male except that we find 14, instead of 15, anterior setigers without swimming setae. Hartman (18) finds that the change occurs in the male "after the 13th segment". She describes the atokous phase for the first time from the Gulf of California and the south coast of Lower California, and epitokes from Ecuador.

Eunicidae

Eunice enteles Chamberlin. Chamberlin (5)

A single example dredged in 15 fathoms at Los Frailes, Mexico, is 40 mm. long and about 3 mm. wide at the widest region of the body. Branchiae begin on the 4th setiger with one filament, increase to a maximum of five filaments at the 9th, and soon thereafter decrease gradually until there are only two at the 25th setiger. There is only one filament on the 30th setiger and this condition persists for only a few segments. Thereafter only the dorsal cirrus remains to the end of the body. These branchial characters lead us to attribute the specimen to *E. enteles*, rather than to *E. antennata* Savigny, which it resembles in other respects. Hartman (19) believes these two species to be synonymous and it is possible that the differences noted may be a matter of age. *E. enteles* is recorded previously from the west coast of Mexico by Rioja (31).

Arabella semimaculata (Moore). Moore (27, as *Aracoda*), Berkeley and Berkeley (2)

A single specimen from Turtle Bay, Lower California, collected on a rocky shore, is 160 mm. long and about 4 mm. wide. The species is recorded widely from California. Hartman (19) says it extends south to Peru.

Lumbrinereis latreilli Audouin and Milne-Edwards. Fauvel (12), Hartman (19)

An example of this species dredged in 15 fathoms at Los Frailes, Mexico, is in three pieces. A cosmopolitan form.

Cirratulidae

Cirriformia (*Audouinia*) *luxuriosa* Moore. Moore (26, as *Cirratulus*); E. and C. Berkeley (2)

Two specimens from Turtle Bay, Lower California, both collected intertidally under rocks. One is 60 mm. long and about 6 mm. wide, the other slightly smaller. The species is widely recorded from California. Rioja lists it from Lower California (31, 33).

Caulleriella alata (Southern). Southern (36, as *Chaetozone*), E. and C. Berkeley (4)

Two small specimens, dredged in 15 fathoms at Los Frailes, Mexico. Both are small, poorly preserved, and each is in two parts. The incidence of bidentate crotchets with wings in the most anterior neuropodia suggests the species *alata* which we have identified in material from Pacific Grove, California (unpublished).

Sabellariidae

Idanthyrsus pennatus (Peters). Johansson (21), Okuda (28)

A specimen from coral at Santiago Bay, Mexico, 40 mm. long, excluding the caudal region. The species is recorded widely from coral reefs.

Sabellidae

Branchiomma mushaensis Gravier. Monro (24)

A specimen collected at Ceralbo Island, Gulf of California, labelled as having been taken "from the shell of a living pearl oyster" is typical of this species. It has been recorded previously from California (2) and from the Gulf of California (15, 33, as *Megalomma*).

Dasychone cingulata Grube. Johansson (21, as *Branchiomma*), Okuda (29)

Several specimens taken from the bait-well of the M. V. May-Queen, the vessel from which the material dealt with in this paper was collected. The species is widely distributed in warmer seas.

Serpulidae

Hydroides uncinata (Phillipi). Fauvel (13), Ehlers (9, as *Eupomatus*)

Specimens varying from 10 to 15 mm. in length, collected at Turtle Bay, Lower California, under rocks. The species is recorded by Treadwell (37, as *Eupomatus*) and by ourselves (2) from California, and from the Panama region by Monro (24).

Pomatoceros minutus Rioja. Rioja (31, 33)

Several specimens from floating seaweed at Abreojo Point, Lower California. The species has been recorded from a number of Mexican stations by Rioja.

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THE MEASUREMENT OF SPRUCE BUDWORM POPULATIONS AND MORTALITY DURING THE FIRST AND SECOND LARVAL INSTARS[1]

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Abstract

Two methods of assessing II-instar budworm populations in hibernacula are presented and a general comparison shows that the collection of foliage samples in the spring and sampling the whole branch as a unit is an acceptable technique. This population assessment permits the partitioning of the total population reduction from the I- to the III-instar larval stage into I-instar dispersal loss in the fall and II-instar dispersal loss in the spring. Estimates of these losses are presented and other mortality factors in operation during this age-interval are discussed.

Introduction

This paper is one of a series of papers (8, 9, 10, 11, 12, 13) on the techniques used in studies on the spruce budworm, *Choristoneura fumiferana* (Clem.), on the Green River Watershed, New Brunswick.

The life history of the budworm, including mortality and survivorship curves applicable to the Green River area, has already been presented (12) and only a brief résumé is needed here. The budworm oviposits during the latter part of July and early August and eclosion is complete by the latter part of August. The I-instar larva, with a few minor exceptions, spins a hibernaculum, molts, and overwinters. In May of the following year the II-instar larva emerges from the hibernaculum and begins the first feeding activity. The age-interval from eclosion to establishment of the II-instar in feeding sites covers a greater part of the life cycle. It is also a period when mortality is consistently high, due largely to the effects of dispersal by air currents. This source of mortality, hereafter referred to as dispersal or dispersal loss, occurs in two periods: (*a*) I-instar dispersal in the fall, and (*b*) II-instar dispersal in the following spring.

Estimates of total dispersal loss for these two periods are calculated conventionally from the difference between I-instar larval counts (egg counts less egg mortality) on a cluster of trees and III-instar counts on the same trees in the spring. Replicated data of this type are available for permanent study plots differing in stand type in the Green River area and have been briefly referred to by Morris *et al.* (14).

The object of the present study is to assess II-instar larval populations in hibernacula and thus to be able to separate the relative effects of fall and spring dispersal on a population. The technique was first proposed to provide mortality data for life tables (12) even though it was realized that estimates of total dispersal would be adequate for an analysis of the effect of

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early larval dispersal on natural populations of the spruce budworm. However, a knowledge of II-instar populations can be of value and such data are used in the western United States to predict the expected degree of defoliation in a stand. Although similar predictions in Canada are made from egg counts (10), with a greater time lapse between prediction and the actual event, egg counting is the more tedious sampling procedure.

Another aspect of this problem, and one that has not been closely followed in a natural population, is the effect of climate on spring and fall dispersal. The role of climate in the initiation of budworm outbreaks is well documented (4, 19). However, the present knowledge of the mechanism through which climate affects natural budworm populations is mainly confined to its effect on moth transport and fecundity (4, 5). Additional evidence may be obtained from an intensive study of specific weather factors on I- and II-instar dispersals, and this of course requires a specific estimate of loss for each dispersal period. A background to such an investigation is already available in the intensive laboratory studies of budworm behavior in relation to light, temperature, and a gradient of evaporation (15, 16, 17, 18).

Sources of Mortality within the I and II Instars

It is convenient to group the total mortality within the I–III-instar age-interval under the term 'dispersal' yet it is known that mortality factors unrelated to air dispersal are in operation. This secondary complex of factors is included in the following outline of mortality factors affecting I- and II-instar budworm larvae.

Age-interval	Probable mortality factors	
I-instar (at egg site)	(*a*) Air dispersal to non-host material (*b*) Predation (*c*) Failure to spin hibernacula (*d*) Diapause-free development	Fall 'dispersal' loss
II-instar (in hibernacula)	(*a*) Loss of hibernacula (*b*) Mortality within hibernacula	
(emergence from hibernacula)	(*a*) Air dispersal to non-host material (*b*) Predation (*c*) Failure to establish a feeding site	Spring 'dispersal' loss
III-instar (within a feeding site)		

Only one of these factors, 'mortality within hibernacula', can be measured exactly for a natural population. The remainder are inferred from either field observations or investigations of field populations as noted in the following subsections.

Air dispersal of larvae.—Three processes may occur as the direct result of dispersal, if a discrete population within a stand is considered: (*a*) redistribution of the population within the stand, (*b*) spread of part of the population to adjoining stands (14), and (*c*) wastage of a part of the population on a non-host material. Of these three processes, wastage is probably the ultimate fate of a major portion of the population as a result of spring and fall dispersal.

Failure to spin hibernacula.—Experiments in which I-instar larvae are provided with suitable sites for spinning hibernacula show that approximately 4% fail to spin (Table I). Egg masses for these experiments were collected at random and placed in small containers along with balsam fir, *Abies balsamea* (L.) Mill., flower bracts. This material was then stored in an insectary and the larvae allowed to emerge and spin under conditions of natural light and temperature. Harvey (6) states that non-spinning appears to be an inherited character and draws the tentative conclusion that "there may be two types of non-spinning individuals—those that do not spin under any conditions and those that spin a hibernaculum only when the photoperiod is shorter than 15 hours."

TABLE I

PER CENT OF I-INSTAR SPRUCE BUDWORM THAT FAIL TO SPIN HIBERNACULA

Year	Total I-instar larvae	Per cent failed to spin
1950	3944	4.4
1951	2067	6.1
1953	1000	3.3
1955	1326	1.8
Total	8337	
Mean		4.3

Diapause-free development.—The absence of diapause among spruce budworm larvae was first noted by Harvey (6). In laboratory-reared material he found that larvae would leave the hibernacula soon after the second molt and begin to feed and that these larvae reached maturity in the laboratory with no apparent ill effect. Diapause-free development, as such, has not been noted in the present study although unaccountable differences have been found in larval counts before and after the spinning of hibernacula. In laboratory experiments this difference averaged 15%, as will be shown below, but dissections did not show that it resulted from the evacuation of hibernacula which is apparently associated with absence of diapause. Harvey, however, calculated an average incidence of non-diapause larvae of 3.4% in collections from Eastern Canada and concluded that a very low level of non-diapause behavior probably occurs naturally. Absence of diapause would result in larval mortality in a natural population but its incidence is apparently so low that it need not be considered an important mortality factor in a natural population.

Mortality within hibernacula.—This mortality refers to the percentage of the population that successfully spins hibernacula but dies during the overwintering period. Table II shows that it ranged from 9 to 22%, indicating relatively little variation over nine generations in one area. There is no evidence to suggest that mortality within hibernacula varies with stand type,

but variations due to hibernation site have not been tested. The results in Table II are based on the dissection of staminate flower bracts, and larvae overwintering under lichen mats, bud scales, and rough bark have not been studied. Larvae for these dissections were obtained from both natural populations and from stock that spun hibernation sites while in the laboratory and were then overwintered in the field. In both cases, however, dissections were made in the spring just before emergence from hibernacula, when mortality was complete and dead and living larvae were more readily separated.

TABLE II

ESTIMATES OF PER CENT MORTALITY WITHIN HIBERNACULA

Year	Total hibernacula dissected	Per cent mortality
1948–49*	139	9
1949–50	164	15
1950–51	736	13
1951–52	1028	16
1952–53†	170	17
1953–54	202	13
1954–55	290	21
1955–56	287	15
1956–57	126	22

*1948–1951: experimental populations overwintered in the field.
†1952–1956: material collected from natural populations.

Loss of hibernacula.—It is not improbable that a number of hibernacula are destroyed during the winter by physical and biotic factors, particularly those spun in the more exposed sites. This loss would be difficult to assess in a natural population. Table III shows the results of two series of experiments designed to assess this loss in experimental populations. In both series a calculated number of I-instar larvae (based on egg counts, less failure to hatch and spin) was allowed to spin hibernacula on shoots containing staminate flower bracts. In one series the shoots were overwintered indoors; in the other, the shoots were overwintered in the field. In both series the actual number of larvae dissected from hibernacula was less than the expected 100%, averaging 15% less for the indoor population, and 31% less for the field population. Although the mean difference cannot be interpreted as a real loss in the field because of the unexplained loss in the indoor population, some possible explanations for the greater loss in the field population can be listed. These include: (*a*) predation by chickadees and nuthatches which might destroy all trace of the hibernaculum, (*b*) spinning of a very 'weak' hibernaculum which would erode before spring, and (*c*) experimental error, so that actual laboratory loss is 0% and field loss is $31-15=16\%$. The last possibility is supported by dissections made only 2 weeks after spinning, which still showed a 15% loss. Even very weak hibernacula could hardly erode in this period.

TABLE III

A COMPARISON OF THE APPARENT 'LOSS' OF LARVAE IN HIBERNACULA FROM EXPERIMENTAL POPULATIONS OVERWINTERED IN THE FIELD AND IN THE LABORATORY

Experiment	Expected number of II-instar larvae in hibernacula (September)*	Actual number of II-instar larvae dissected from hibernacula (May)	Per cent 'loss'
Field			
1951	1168	736	37
1951	459	290	37
1952	1409	1028	27
1954	655	454	31
1955	812	603	26
Total	4503	3111	
Mean			31
Laboratory			
1951	899	738	18
1951	479	437†	9
1952	848	754	11
1953	719	610†	15
1954	1597	1282	20
1955	1326	1140‡	14
Total	5868	4961	
Mean			15

*Egg counts less failure to hatch and failure to spin.
†Visual count, not dissection, of hibernacula approximately two weeks after spinning.
‡Dissection of II-instar larvae from hibernacula approximately two weeks after spinning.

Predation.—No estimates of predation on I- and II-instar larvae are available. Spiders are active in the fall and again early in the spring but laboratory experiments indicate that they do not readily attack I- or II-instar larvae. Thus, since these larvae are only exposed for a short time, either before they spin a hibernaculum or establish a feeding site, it is probable that very few fall prey to spiders or other predators.

Failure to establish a feeding site.—Experiments designed to test the time required for II-instar larvae to establish feeding sites on different types of foliage have been carried out under laboratory conditions. In cases where whole branches were used and a multiple choice of feeding sites existed, all the larvae became established. In other experiments where individual larvae were caged with one type of foliage, failure to settle varied with the type of foliage, but did not exceed 5% even on unfavorable food (3). These results indicate that under natural conditions where a choice of food is available, there is little reason to suspect that an appreciable percentage of II-instar larvae fail to establish feeding sites in the spring.

Summary.—Mortality factors other than wastage from dispersal are present during the I- to III-instar age-interval and are difficult to assess in a natural population. However, these secondary factors, with the exception of 'mortality within hibernacula' which is tabulated separately in a spruce budworm life table (12), obviously account for a very small proportion of the mortality

in relation to the dispersal factor. Further, they are unrelated to host density and their significance as mortality factors effecting changes in annual population trend are therefore slight. Thus, the practice of combining these factors with the wastage factor under the term 'dispersal' seems to be justified.

Location of Hibernation Sites

The preferred overwintering site of spruce budworm larvae has been investigated by other workers and conflicting results obtained. Both Bess (1) and Blais (2) found that I-instar larvae show a preference for staminate flower bracts as overwintering sites. Jaynes and Speers (7), however, found no preference for staminate bracts over annular bud scales. These conflicting results are partly due to the basic difficulty in setting up a sampling design that measures the density of the population on different parts of a branch. The surface area of the bark both on the trunk and the branches could be considered the sampling universe for hibernacula but it is very difficult to measure this surface in order to equate differences in sample size, particularly when staminate flower bracts are present. Blais measured the length of twig and expressed the population per 100 in. of foliated twig. Jaynes and Speers dissected the whole branch and expressed their results as percentages of the total population found.

The location of hibernation sites on mature balsam fir was assessed in the present study. Each branch from four crown levels was roughly divided into four sections on the assumption that a balsam fir branch is roughly the shape of an isosceles triangle and can be divided into sections so that each section contains shoots of approximately an equal number of years growth. Thus, Section 1 comprised the peripheral shoots at the two sides of the triangle. These were clipped from the branch. Section 2 was the next layer of shoots, Section 3 the next, and Section 4 the basal portion of the main stem of the branch. The number of internodes and clusters of staminate flower bracts were counted in each section. Five trees were sampled in this manner and the results considered in two ways: (*a*) the actual number of larvae that emerged from each branch section, and (*b*) the population density per 100 internodes on each branch section.

A total of 8938 larvae emerged from the samples and Table IV shows the percentage distribution of these larvae on Sections 1 to 4 as 33%, 27%, 27%, and 13%, respectively. Although more larvae emerged from Section 1 there is no concentration at the peripheral portion of the branch as found by Jaynes and Speers, nor is there any apparent influence of staminate flower bracts on the distribution of the population. Table V shows little difference in the percentage distribution on flowering and non-flowering branches, and further, the distribution of flowers and larvae per branch section shows no apparent correlation.

No definite evidence of a preferred hibernation site was found on the basis of population density per section. The density was calculated per 100 internodes after an attempt was made to compensate for differences in shoot

TABLE IV

LARVAL EMERGENCE PER BRANCH SECTION TABULATED AS A PERCENTAGE OF THE TOTAL EMERGENCE FROM THE TREE SAMPLE. PLOT G9, 1952

Tree	Branch section 1	2	3	4	Total emergence
6	21	30	25	24	1535
7	38	36	20	6	1220
8	39	24	23	14	1456
9	32	23	34	12	3053
10	39	27	25	9	1674
Total					8938
Mean	33	27	27	13	

TABLE V

PER CENT DISTRIBUTION OF THE LARVAL POPULATION ON 'FLOWERING' AND 'NON-FLOWERING' BRANCHES. PLOT G9, 1952

	Branch section 1	2	3	4	Total emergence
Branches without flowers*	37	25	24	14	1149
Branches with flowers	33	27	27	13	7789
Per cent distribution of flowers	65	27	8	<1	

*Small branches from level A.

TABLE VI

MEAN POPULATION PER 100 INTERNODES FOR BRANCH SECTIONS, CROWN LEVELS, AND TREES AND THE ANALYSES OF VARIANCE OF THESE DATA. PLOT G9, 1952

Trees	Levels	Sections
1. 368.9	A. 262.4	1. 336.3
2. 242.5	B. 510.1	2. 273.9
3. 225.5	C. 476.4	3. 496.0
4. 508.7	D. 390.9	4. 533.6
5. 294.2		

Source of variance	Sum of squares	Degrees of freedom	Mean square	Error term
(1) Trees	3,330	4	832.5*	(4)
(2) Levels	1,829	3	609.7*	(4)
(3) Sections	2,331	3	777.0*	(4)
(4) Error	10,175	69		
(5) Trees × levels	1,246	12	103.8	(8)
(6) Trees × sections	2,894	12	241.2	(8)
(7) Sections × levels	1,365	9	151.7	(8)
(8) Trees × levels × sections	4,670	36	129.7	

*Significant within the 1% level.

diameter. Shoot diameters were measured along the main stem of the branch and mean differences in size from Sections 1 to 4 were found to be in the ratio of 1:2.2:3.5:4.7, respectively. The number of internodes per section was adjusted by this ratio with the assumption that shoot lengths in all sections were approximately equal.

Table VI shows the mean population densities and the analysis of variance for five trees, four crown levels, and four branch sections. Significant differences between trees and between levels are not unexpected (11). No precise interpretation can be placed on the significant difference between sections since the sampling unit is so ill defined. However, the results do show that the unit of collection for assessing overwintering populations in hibernacula must be the whole branch and that staminate flower clusters have little influence on the distribution of the overwintering population.

Population of II Instar in Hibernacula

Since hibernacula are too small and well concealed to be counted accurately by the visual examination of foliage sample, two methods of collecting and counting the emerged II-instar larvae were tested. These are outlined separately under Method I and Method II.

Foliage and Bark Samples

Foliage samples were collected in accordance with the sampling principles outlined by Morris (11). Sampling points were chosen in the vicinity of ecological plots where egg and III-instar larval counts were regularly obtained. The trees were felled, the crowns divided into vertical levels labelled A, B, C, and D, and whole branches, including a small ring of trunk bark at the base of each branch, were removed from the mid-point of each level. Sampling intensity per tree was either four branches in the ratio of 1A, 1B, ½C, and ½D or six branches in the ratio of 2A's, 2B's, 1C, and 1D. A cluster of 10 codominant balsam fir was sampled per plot.

Samples of trunk bark were also collected from some trees in the cluster. Sampling points were confined to the middle of the clear portion, and the middle of the crown portion of the trunk. Total surface area of bark was calculated on the assumption that the trunk is cone-shaped, and population per bark sample was converted on this basis to population per trunk. On some trees the bark was carefully peeled from the trunk, on others, whole sections of the trunk were cut and sampled.

Method I

Foliage and bark samples were obtained in September after the overwintering population was settled in hibernacula. Each branch was measured (11), clipped into small sections, and placed in a paper bag on which tree number and crown level were recorded. The samples were placed in a controlled-temperature room, first at 42° F. for a period of 5 days, and then at 32° F. for a period of approximately 26 weeks. At the end of this time the temperature was raised to 42° F. for a period of 5 days. The needles that

had dried up and dropped from the foliage were removed and the samples thoroughly sprayed with water. They were then placed in a rearing room at 72°–76° F. and approximately 70% R.H., well sprayed daily with water for 3 days, dried on the 4th day, and then placed in an emergence cage.

Two types of emergence cage were used; one a light metal tin of approximately 3-qt. capacity, the other a cylindrical waxed-cardboard container with a 1-gal. capacity. Both had tight-fitting covers. In the former type a ¼-in. hole was bored near the rim of the cover. A small block of wood with a hole 1 in. in diameter was fastened to the cover so that the two holes were in alignment. A short piece of heavy rubber tubing was fitted to the block of wood and, in turn, a shell vial inserted into the rubber tubing. Thus, larvae had access to the vials and the vials were easily removed and replaced. Screw-cap vials were used on the cardboard container. A ¼-in. hole was bored in the cap and this was sealed to the cover near the rim.

The emergence cages were loosely filled with the foliage samples or bark samples and the covers sealed with masking tape. They were then placed on their sides in a holding rack with the vials uppermost and pointed toward a bank of lights acting as a light source. The lights were in operation approximately 10 hours per day. The larvae, attracted into the vials by the light, were collected and counted periodically during the emergence period.

Control experiments, to assess the percentage emergence from hibernacula, were set up in the following manner. Balsam shoots with staminate flower bracts were placed in bottles with a known number of budworm eggs. The bottles were sealed with two layers of tightly-woven cotton and partially covered so that only the bottom of the bottle and the shoots were exposed to direct light. Approximately 100 larvae spun hibernacula for each control experiment. The shoots were then mixed with 'sterile' foliage so that the sample equalled an average field sample in size. Control samples were treated in the same manner as field samples during the storage and emergence periods.

Method II

This method differs from Method I in that the foliage is collected in the spring after overwintering under natural conditions and, further, that the whole branch is sampled as a unit. The technique of obtaining larvae from whole branches was first devised at the Forest Insect Laboratory, Sault Ste. Marie, Ontario. In the present study, foliage samples were collected early in May since emergence of II-instar larvae in the field normally occurs in the 2nd or 3rd week of May in the Green River area. Whole branches were collected and measured as in Method I, with a sampling intensity of six branches per tree. The branches from one tree were tied together and wrapped with paper towelling to make a cone-shaped covering of paper with the open apex at the butt end of the branches. The 'wrapped' sample was then suspended under a strong light. The larvae were collected as they crawled on the paper; those that dropped were caught on a sheet of paper placed

beneath the sample and ringed with 'tanglefoot'. The suspension string was also ringed with tanglefoot. The samples were sprayed periodically with water during the emergence period.

No control experiments were set up to assess percentage emergence from hibernacula as in Method I. Instead, shoots with staminate flower bracts were clipped from the branches and examined for hibernacula after emergence was complete. Percentage emergence was calculated on the ratio of hibernacula showing successful emergence to the total number of hibernacula found. This method of assessing emergence was also used in one instance in Method I but it is time-consuming, particularly at low population levels.

Bark samples were obtained by cutting sections approximately 3 feet long from the trunk of a number of sampling trees. Two sections per tree were collected, one from the mid-point of the crown portion of the trunk, the other from the mid-point of the clear portion of the trunk. Nails were driven into the section so that it could be propped up horizontally on a table. Paper, ringed with tanglefoot, was placed under the section and the larvae collected as they wandered over the bark or dropped to the paper.

Results

In Method I initial emergence from hibernation occurred approximately six days after the foliage was removed from storage at 42° F. In Method II, where the foliage was collected early in May, initial emergence usually occurred 1 or 2 days after the foliage was brought indoors. In both methods the emergence period lasted about seven days with the bulk of the population emerging over a 5-day period.

The total number of larvae emerging from a tree sample was counted and the population calculated per 10 sq. ft. of branch surface. In Method II portions of the tree sample were examined after emergence to assess the number of larvae that established feeding sites, in preference to coming out on the paper and the population adjusted by this figure. Finally, in both methods, the population was adjusted by the percentage emergence figure, obtained either from the control experiments or dissection of hibernacula to give the total population of hibernacula per 10 sq. ft. of branch surface.

In the period 1952–57, 24 trees were sampled using Method I and 99 trees using Method II. The total number of larvae counted per plot ranged from approximately 400 to 10,000. The percentage emergence of larvae from hibernacula ranged from 59 to 85% in Method I and from 78 to 83% in Method II.

It should be noted, however, that larvae hibernating on the trunk are not included in the estimation of mean population per plot. There are two reasons for this: (*a*) it simplifies the technique of assessing the overwintering population, and (*b*) the population on the trunk is only a negligible part of the total population on the tree. Table VII shows that the trunk population is less than 1% of the total population in open, mature stands and approximately 4% in dense stands. In this table the branch population per tree is estimated from the relationship of D.B.H. to total branch surface area (11).

The trunk population data for the open stands are based on bark samples peeled from the trunk and sampling intensity is therefore low. In 1956, however, two trunk sections per tree were collected from each of eight trees giving a sampling intensity of approximately 12% of the total bark surface.

TABLE VII

COMPARISON OF II-INSTAR POPULATIONS ON THE TRUNK AND BRANCHES

Year	Stand	Larval population on trunk	Larval population on branches	Per cent of population on trunk
1952	Open, mature	417	60,371	0.7
1953	Open, mature	470	60,736	0.8
1954	Open, mature	102	10,660	0.9
1956	Dense, middle-aged	684	17,980	3.7

TABLE VIII

FALL DISPERSAL LOSS PER TREE, PLOT K3, 1956. EGG AND LARVAL COUNTS BASED ON SAME 10 TREES

Tree	Eggs per 10 sq. ft.	I-instar per 10 sq. ft.	II-instar per 10 sq. ft.	Per cent fall dispersal loss	Deviations from mean dispersal loss
1	2733	2377	804	66	+ 7
2	1414	1230	580	53	− 6
3	2992	2603	811	69	+10
4	2279	1983	419	79	+20
5	1988	1730	759	56	− 3
6	1881	1636	760	56	− 3
7	1930	1679	669	60	+ 1
8	900	783	382	51	− 8
9	2259	1965	935	52	− 7
10	1909	1661	869	48	−11
Mean				59	

TABLE IX

FALL DISPERSAL LOSS PER TREE, PLOT G12, 1957. EGG AND LARVAL COUNTS BASED ON SAME 10 TREES

Tree	Eggs per 10 sq. ft.	I-instar per 10 sq. ft.	II-instar per 10 sq. ft.	Per cent fall dispersal loss	Deviations from mean dispersal loss
1	276	210	61.0	71	− 3
2	663	504	77.9	85	+11
3	214	163	55.8	66	− 8
4	443	337	75.4	78	+ 4
5	141	107	29.9	72	− 2
6	267	203	40.5	80	+ 6
7	318	242	51.8	79	+ 5
8	140	106	41.7	61	−13
9	609	463	86.7	81	+ 7
10	263	200	59.1	70	− 4
Mean				74	

The removal of whole sections is the better sampling procedure and this may account for the higher estimated trunk population in a dense stand. Apparently bark texture is one of the factors limiting the trunk population since in the Western United States, on hosts with a rough bark, the proportion of the population on the trunk is much higher than the above estimates for balsam fir which has a relatively smooth bark.

The degree of precision with which the mean population can be measured by Method II can be indicated approximately from the limited data at hand. For example, the population data obtained in 1956 from five plots were transformed on the basis of $y=\log_{10}(x+1)$ and the intertree variance calculated for the 10 trees per plot (11). These variances, when plotted over the mean, fall within the pattern of variance for larval and pupal counts shown by Morris (11, Fig. 12) indicating that the variance for II-instar counts may be similar to the variance for III-instar and pupal counts. If so, the required number of sampling units per tree, and trees per plot, outlined by Morris to define larval and pupal population means with a set precision may also be applicable to II-instar population. A further example (Tables VIII and IX) of the degree of precision obtained by this method of sampling is shown in the fall dispersal losses for two plots where an opportunity was afforded to make I- and II-instar counts on the same 10 trees. The deviations from the mean dispersal loss are not excessive and indicate that 10 trees may define the loss with an acceptable reliability.

Fall and Spring Dispersal Losses

Table X shows the results of experiments to assess II-instar populations and partition total dispersal into fall and spring components for the period 1952–57. Fall dispersal is calculated on the mean difference between I- and II-instar larval counts, and spring dispersal on the mean difference between II-instar counts, less mortality in hibernacula, and III-instar larval counts. Only the egg (I instar) and III-instar counts are obtained from the same cluster of trees.

The aspects of dispersal loss that are particularly related to the population dynamics of the budworm are the degree of variation in dispersal from year to year in any given stand, and the difference in dispersal between different types of stands. These problems can be investigated on the basis of the combined dispersal loss of I- and II-instar larvae and some conclusions have already been presented (14). The results of partitioning total dispersal into fall and spring components do not alter these conclusions but do suggest problems for further investigation. For example, a mean fall dispersal loss of 64% may be calculated from Table IX. Deviations from this mean are not excessive, at least in comparison with spring dispersal where the population change ranges from a 71% loss to gains resulting from immigration. Larval movement may therefore be more extensive in the spring, and weather conditions at that time may play an important role.

TABLE X

ESTIMATES OF FALL AND SPRING DISPERSAL LOSSES, 1952–57

Year	Plot	Stand type	Per cent fall dispersal loss	Per cent spring dispersal loss
1952	K1	Open, mature	67	54
1953	G9	Open, mature	58	70
1954	K1	Open, mature	48	71
1955	G13	Open, mature	57	49
1955	G12	Dense, middle-aged	49	18
1956	G12	Dense, middle-aged	69	58
1956	K3	Dense, middle-aged	60	43
1956	K2	Dense, middle-aged	63	49
1956	I2	Open, mixed-wood	62	+36
1956	I3	Dense, middle-aged	70	+ 2
1957	G12	Dense, middle-aged	77	
1957	K2	Dense, middle-aged	71	71
1957	I3	Dense, middle-aged	82	

Dispersal loss is also related to the presence of staminate flowers for these affect the rate of establishment of II-instar larvae on balsam fir. However, Greenback *et al.* (3), having investigated this problem in a diversified forest in both flowering and non-flowering years, set forth the theory that the net effect of staminate flowers on dispersal is minor in comparison with such factors as topography, forest continuity, stand density, and weather conditions.

The effect of spring dispersal on that portion of a population parasitized by *Apanteles fumiferanae* Vier. and *Glypta fumiferanae* (Vier.) has also been studied. These parasites attack I- and II-instar larvae in the fall. Trends in percentage parasitism obtained from dissections of II-instar larvae emerging each day over a 4-day period indicate that parasitized individuals leave hibernacula during the latter part of the emergence period. However, there is no differential mortality within the host population during the dispersal period, since there is little variation in estimates of percentage parasitism based on dissections of II-instar larvae emerging from hibernacula and later estimates based on III-instar larvae in feeding sites.

Discussion

The question of the relative merits of the two methods of assessing II-instar population in hibernacula cannot be answered categorically. Accessibility of the study plots early in the spring and laboratory facilities at a field station for the treatment of foliage samples may dictate the method to be used. However, if a choice is available, Method II is the better technique. The primary disadvantage of Method I is that the foliage is not overwintered under natural conditions. Further, the samples require excessive handling before the larvae are recovered from the foliage, and special facilities are needed for overwintering storage. These facilities may also limit the number of samples that can be handled in any one year. Another difficulty is the

treatment of foliage before it is placed in emergence cages. If the foliage is too wet, fungus growth soon develops and the sample must be discarded. Conversely, mortality in hibernacula increases if the foliage is too dry.

The advantages of Method II are that the population overwinters under natural conditions and no special facilities are needed to recover larvae from hibernation. A disadvantage is that, after emergence, a portion of the foliage must be sampled for larvae that establish feeding sites. This is particularly true when staminate flowers are present. In general, however, the technique is an efficient means of estimating II-instar budworm populations in hibernacula.

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A NEW HOST OF ENTAMOEBA INVADENS RODHAIN, 1934[1]

EUGENE MEEROVITCH

Abstract

A strain of *Entamoeba invadens* has been isolated for the first time from a natural infection in a painted turtle, *Chrysemys picta*. Infection experiments with this strain have corroborated the earlier observations on the high pathogenicity of *E. invadens* in snakes, but not in turtles. This, together with the fact that *E. invadens* in the past had been isolated only from captive snakes, which might have become infected in captivity from turtle carriers, suggests that *E. invadens* is a natural, non-pathogenic parasite of turtles.

Entamoeba invadens (5) has been implicated as the etiological agent of snake amoebiasis, a condition which is characterized by a severe ulceration of the tissues of the digestive tract and an invasion by the amoebae of almost all the internal organs. Snake amoebiasis as a rule is much more severe than human amoebiasis, caused by *Entamoeba histolytica*. Extraintestinal foci and lesions of a more generalized nature appear earlier in the disease and the infection culminates in death, after the parasites have invaded practically every organ of the animal (4).

Materials and Methods

Painted turtles, *Chrysemys picta*, captured in the vicinity of Ste. Anne de Bellevue, Quebec, were kept in separate glass aquaria, 12 × 8 × 8 in., in which they could move in and out of water. The turtles were offered lettuce, cabbage, and other green vegetables, but they fed irregularly and in small amounts.

Garter snakes, *Thamnophis sirtalis*, and water snakes, *Natrix sipedon*, captured in the vicinity of Ste. Anne de Bellevue, Quebec, or obtained from a dealer in Toronto, Ontario, were kept in individual gallon jars with perforated metal screw caps. Because the snakes could not be induced to take any food offered to them, they were periodically force-fed with a mixture of milk, whole raw egg, and cod-liver oil.

The following abbreviations, followed by serial numbers, are used in the text to represent the experimental animals:

Thamnophis sirtalis	T
Natrix sipedon	N
Chrysemys picta	Tur

E. invadens was cultivated with a mixed concomitant bacterial flora in serial transfers every 2 weeks, in Balamuth's buffered egg-yolk infusion medium with rice starch (1).

Bacteria-free cultures of *E. invadens* were established by placing pieces of aseptically excised infected livers of snakes into a sterile medium consisting

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of dilute bovine serum (10% by volume in 0.75% saline) and antibiotics (penicillin 500–1000 I.U. per ml., and streptomycin 500–1000 μg. per ml.). Bacteria-free cultures were maintained by transferring the trophozoites into the same liquid medium in which the snake liver was replaced by sterile fresh hamster liver.

Whenever a diagnosis of infection had to be made by culture technique, samples of faecal material or of tissues were placed in the culture medium.

For the histological demonstration of the parasites, tissues of reptiles were fixed in Bouin's fluid or Zenker's formalin, embedded in paraffin, sectioned, and stained.

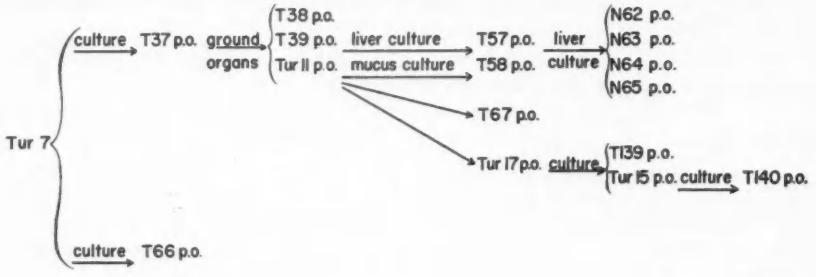
Inoculation of snakes was made by introducing the material into the digestive tract through male catheter tubing attached to the barrel of a syringe. In the case of turtles the material was forced through a blunted glass pipette attached to a rubber bulb. Snakes to be inoculated with amoebae were examined three times for natural infections before they were considered to be amoeba-free. Pre-inoculation and post-inoculation examination of snakes was done by expressing faecal material by applying pressure on the abdomen, while the snakes were held firmly by their necks. Turtles were examined before and after inoculation with amoebae by withdrawing some intestinal contents through the cloaca with a blunted pipette.

Experimental

(Fig. 1)

During the course of some work on the infectivity of *E. invadens* to different classes of reptiles, one painted turtle, *Chrysemys picta*, No. 7, was discovered to be naturally infected with a species of *Entamoeba*.

Two cultures were made of the material on Balamuth's medium. Eleven days later one of them was weakly positive for trophozoites and the other had a heavy growth of trophozoites and a large number of cysts. The rich



Tur – adult *Chrysemys picta*; T – adult *Thamnophis sirtalis*;
N – adult *Natrix sipedon*.
p.o. – *per os* inoculation.

FIG. 1. Isolation and history of the I.P.2 strain of *E. invadens*.

culture was transferred into four subcultures, only one of which became positive. The latter was subcultured into two other cultures. In 5 days these cultures were seen to be positive for trophozoites and cysts. One of them was concentrated by centrifugation and administered per os to T37. The other was again transferred but none of the subcultures became positive.

Examination of T37 6 days after inoculation revealed numerous trophozoites in the faecal material which contained many loose epithelial cells. The snake was found dead 6 days later. On macroscopic examination it was seen that the large intestine and the liver presented a typical pathological picture of amoebiasis, and amoebae were seen in large numbers when fresh smears of these organs were examined under the microscope. Some cultures were started from the liver, but after five passages in vitro the strain was lost.

The large intestine and the liver of T37 were ground in saline and the suspension was administered per os to T38, T39, and Tur11.

Subsequently two more cultures were started from the faecal material of Tur7. Eleven days later they were strongly positive and one was given per os to T66. The other culture was transferred, but after three more passages in vitro the strain was lost.

Nine days after its inoculation T66 passed blood containing amoebic trophozoites. The snake was found dead 10 days later. Its viscera presented a typical pathological appearance; amoebae were seen in the fresh smears of the large and small intestine, liver, kidneys, and heart blood. The subsequent histological examination of the sections of the intestine and the liver revealed the typical necrosis and swelling of the intestinal wall, thrombosis of liver capillaries, and amoebae in both the organs.

Tur7 was found dead 206 days after the first isolation of the amoebae from its intestinal contents. The post-mortem examination revealed neither pathology nor any parasites. Cultures started from intestinal contents and from the liver remained negative for amoebae.

T38 died 7 days after inoculation. There were few amoebae in its intestine, which was not altered. The cultures which were started from it did not become positive. T39 died 5 days after inoculation. Its intestine showed signs of early pathology. Amoebae and cysts, recovered in cultures made from its liver, have been maintained in serial transfers.

T57 was inoculated per os with trophozoites and cysts from one of the liver cultures of T39. It died 21 days later and the signs of typical pathology of amoebiasis were evident in its viscera. Amoebae and cysts from a culture of the liver of T57 were given per os to N62, N63, N64, and N65. The last four snakes were killed 13 days later and bacteria-free cultures of amoebae were started from their livers. Later some of the bacteria-free trophozoites pooled from these cultures were placed in Balamuth's medium seeded with bacteria isolated from the faeces of N62.

The strain thus derived has been maintained in serial transfers and yielded rich harvests of trophozoites and cysts.

Fifty-one days after inoculation, Tur11 passed some mucus, which when examined under the microscope was seen to contain amoebic cysts. The

cysts were not measured at that time, but the mucus was cultured, and the cultures became positive for trophozoites and cysts. These were inoculated per os into T58, which died 22 days later of a generalized, typical amoebiasis.

When Tur11 was killed 66 days after inoculation, cultures were started from the different parts of its digestive tract, liver, and spleen. Only those from the large intestine became positive. T67 was inoculated per os with the material from one of these cultures; it died of typical amoebiasis 12 days later. Material from another culture derived from the large intestine of Tur11 was inoculated per os into Tur17. The latter was killed after 2 days. Positive cultures were derived from its intestinal contents.

Trophozoites and cysts from one of these cultures were given per os to T139 and Tur15. T139 died of typical amoebiasis 20 days later. Tur15 died 17 days after inoculation. Positive cultures were derived from all portions of its digestive tract, but not from the liver. Trophozoites and cysts, pooled from the cultures of Tur15 intestine, were inoculated per os into T140. T140 died 9 days after it had been infected; typical pathology and amoebae were seen in the large intestine and the liver. Cultures made from these organs became positive.

Tur8, which had been proved free of parasitic amoebae by microscopical and culture methods, was force-fed with a portion of the large intestine of snake T149, which contained trophozoites and cysts of the amoebae, isolated originally from Tur7. Snake T150 was placed into the aquarium together with Tur8, 8 days after the latter was infected. The aquarium contained ½ in. of water, but the animals were able to come up on to a dry surface.

Twenty-eight days after the turtle was infected, cysts were observed in a sample of its faeces. On the same day cysts were seen also in the water in the aquarium. A sample of turtle faeces containing the cysts was placed in culture medium, and subsequently a culture of amoebae was obtained from it.

After T150 had been in contact with Tur8 for 35 days its faeces were seen to contain blood, mucus, and amoebic trophozoites. Since it was presumed that the amoebae were *E. invadens*, the snake was isolated from the turtle into a separate jar, and was observed for the development of the disease.

The culture obtained from the faecal sample of Tur8 was administered per os to T151.

T151 died 6 days later and the autopsy revealed typical pathology in the large intestine, as well as amoebae in the digestive tract and the liver.

T150 survived for 10 days after it was removed from the turtle tank. During this period it passed blood and amoebic trophozoites and cysts. The characteristic swelling of the large intestine could be felt for a few days before it died. Post-mortem examination revealed signs of typical snake amoebiasis, with the parasites present in the digestive tract and the liver, which had several discrete abscesses.

Tur8 was killed 2 days after T150 was removed from its tank. No pathology was seen in any portion of its digestive tract, but some amoebic cysts were seen in the lumen of the rectum.

On the basis of its typical pathogenicity to snakes and morphology (600 four-nucleate cysts measured had a mean diameter of 14.93 μ with a standard deviation of ± 1.978 μ), it was concluded that this organism was *Entamoeba invadens*, and the strain was named I.P.2. The difficulty with which it went into culture indicated that it must be a new strain. Once established in vitro, the I.P.2 strain produced rich cultures in bacteria-free media and in media containing bacteria.

Discussion

All the reports of "spontaneous" amoebiasis in snakes were based on observations on captive reptiles (2). There are no data available on the prevalence, or even the existence of this condition in the field. The strains of *E. invadens* under cultivation in different laboratories throughout the world have been isolated from snakes which were originally brought from the tropics, but the possibility of their having become infected with *E. invadens* after capture must not be ruled out. Experimental amoebiasis due to *E. invadens* has been established successfully only in snakes and lizards (2, 7). Attempts to infect turtles with *E. invadens* resulted either in failures or in infections not resulting in any pathological condition (3, 6, 7, 8). Only two turtles were reported to have developed the disease, and as can be judged from the literature, they were inoculated per rectum, so that the amoebae may have been introduced into the tissues through a wound inflicted during the operation (7, 8). The idea that turtles may be the carriers of *E. invadens* and that snakes may become infected by indirect contact with the turtles in places where these animals are kept in captivity, has been expressed (8), but until this time *E. invadens* had not been isolated from a turtle.

The experiments reported above have led to the conviction that turtles and not snakes are the natural hosts of *E. invadens*. A hypothesis attempting to explain why *E. invadens* infections in snakes are accompanied by pathological changes culminating in the death of the host, while the parasitism in turtles may be described as commensal, will be published in another paper.

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THE METAZOAN PARASITES OF THE HETEROSOMATA OF THE GULF OF ST. LAWRENCE

IV. CESTODA[1, 2]

KEITH RONALD[3]

Abstract

Diplocotyle olrikii, *Clestobothrium crassiceps*, *Bothriocephalus scorpii*, and *B. claviceps*; *Scolex pleuronectis*, *Phyllobothrium* sp. (larva), and a tetraphillidean plerocercoid (Cestoda) were identified in a study of 560 specimens of Heterosomata (*Hippoglossoides platessoides*, *Hippoglossus hippoglossus*, *Limanda ferruginea*, *Liopsetta putnami*, *Pseudopleuronectes americanus*, and *Scophthalmus aquosus*) from the Gulf of St. Lawrence area. The distribution of the hosts is indicated.

This is the fourth of the series of papers (17, 18, 19) describing the Metazoa of flatfish of the Gulf of St. Lawrence area. One hundred specimens each of *Glyoptocephalus cynoglossus*, *Hippoglossoides platessoides*, *Hippoglossus hippoglossus*, *Limanda ferruginea*, *Pseudopleuronectes americanus*, *Scophthalmus aquosus*, and 60 specimens of *Liopsetta putnami* were examined. *G. cynoglossus* was free from cestodes, which is difficult to explain in view of the similarity of the habits of this fish to other flatfish of the Gulf, but follows the findings elsewhere in that the literature contains only one reference (16) to a cestode infection of *G. cynoglossus*.

While cestodes are common parasites of fish, only 37 true cestode species have been recorded from the Heterosomata anywhere, and cestodes are poorly represented in this survey. This may be due to incompatability between the cestodes and their benthic hosts, or merely to insufficient knowledge of the parasitic fauna of the Heterosomata.

Live specimens were stained by immersion in Rukhadze and Blazhin's (20) stain for 1 hour, during which they were lifted by the scolex to ensure their death in an extended position. The lactic acid present in the stain killed the cestode during this process. Dead specimens were stained with Mayer's carmalum after treatment with Langenbeck's modification of Kleinenberg's fixative.

Scolex pleuronectis Müller, 1788

S. pleuronectis has been recorded from 18 different flatfish throughout the oceans of the world. It is believed that, when the life histories of several related species of cestodes are known, *S. pleuronectis* will prove to be their larval form but until that time the trivial name has to suffice.

Host: *Hippoglossoides platessoides*,
Hippoglossus hippoglossus,
Limanda ferruginea.

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[2]No. 66, Contributions du Département des Pêcheries.
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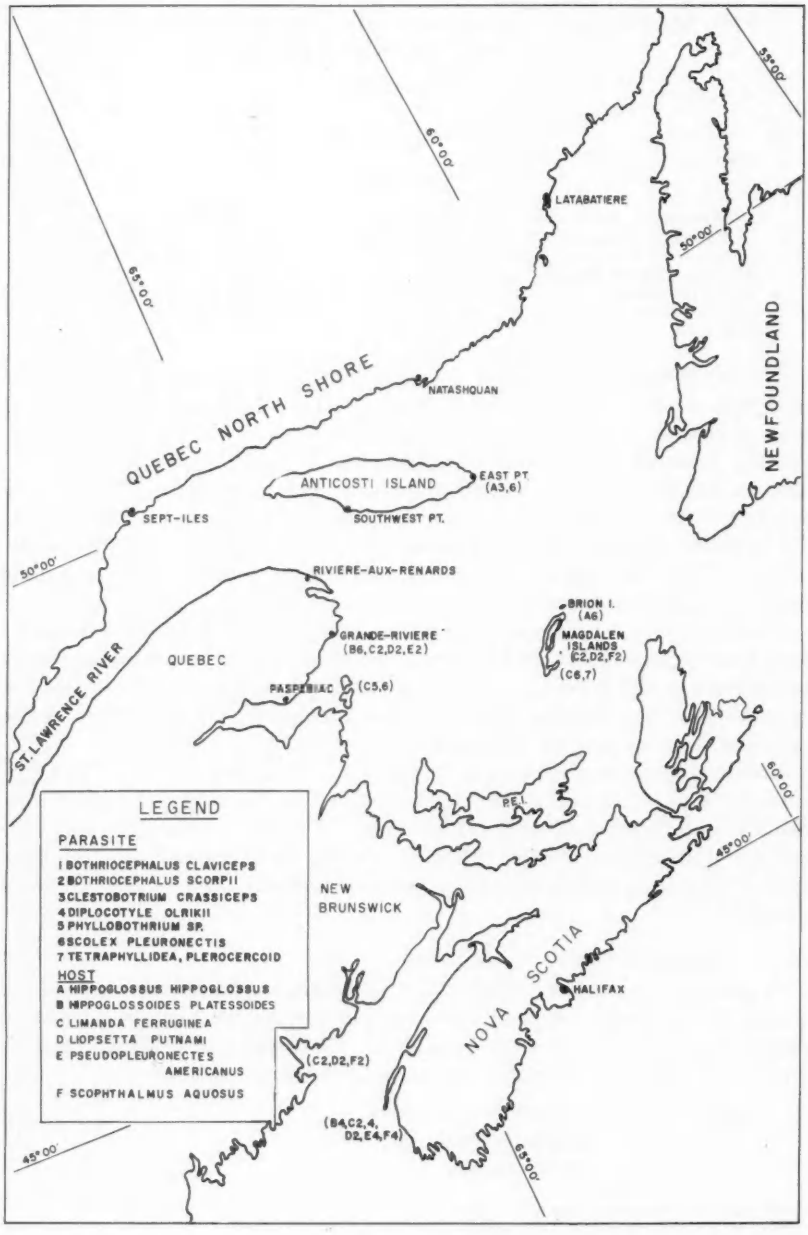

FIG. 1. Distribution of the cestode parasites of the Heterosomata in the Gulf of St. Lawrence region.

Location: Stomach and intestine of halibut (*H. hippoglossus*).
Pyloric caeca and intestine of yellowtail (*L. ferruginea*).
Intestine only of the plaice (*H. platessoides*).
Locality: *H. platessoides*: Grand River.
H. hippoglossus: Brion Island, Magdalen Islands; East Point, Anticosti Island.
L. ferruginea: Miscou Bank; Entry Island, Magdalen Islands.

This parasite has been recorded from the plaice in European waters by Diesing (4), Lönnberg (11), Müller (12, 14), and Nicoll (15); and from American waters by Linton (9). In the present collection only two specimens were found in two plaice captured 1000 meters offshore.

Linton (7) also recorded this parasite (*Scolex polymorphus*) in the yellowtail. It was fairly rare in the present survey; only 15% of the fish carried one or two tapeworms.

Müller (13) and Cobbold (1, 2) reported *S. pleuronectis* from European waters, and the present work is the first record of it in a halibut from North American waters. It was present in numbers of up to 63 in 80% of the fish examined, and with a mean incidence figure of 12.

The larvae do not differ in size or morphological structure from host to host. The length of the entire worm is between 0.8 and 2.4 mm., the scolex measures 0.18 to 0.22 mm. in length by 0.20 to 0.24 mm. at its widest point. The four-lobed scolex carries a thick edged bothridium on each lobe, measuring 0.115 to 0.170 mm. in length. In some specimens the bothridia are divided by a transverse ridge into a small anterior and larger posterior locular. In the 600 specimens examined 50% had a small apical sucker, 0.050 to 0.074 mm. in diameter. These latter specimens would tend to corroborate the results of experimental infections in which adult specimens of *Phoreiobothrium triloculatum* were recovered from sharks fed with infected *Cyoscion regalis* (3). The genus *Phoreiobothrium* was erected for specimens from a shark with *P. lasium* as the genotype (6). At a later date the species *P. triloculatum* was added (8). The genotype was distinguished from the closely related species in the genus *Cylindrophorus* Diesing, 1863, by the presence of an apical sucker. The apical sucker present in about 300 specimens of *S. pleuronectis* suggests that the halibut, yellowtail, and plaice are the intermediate hosts of this parasite, final development occurring in an elasmobranch.

Phyllobothrium sp., larva

Host: *Limanda ferruginea.*
Location: Pyloric caeca.
Locality: Miscou Bank.

The four small larvae recovered differ from *S. pleuronectis* in the lack of transverse ridges, and the globular shape of the bothridia. The body is smaller, 0.79 to 1.05 mm. long by 0.28 to 0.48 mm. wide. The bothridia measure 0.126 to 0.150 mm. in diameter. The small apical sucker measures 0.084 mm. in diameter.

These larvae resemble those described for the genus *Scyphophyllidium* (22), except for the presence of the fifth sucker. A closely related genus, *Myzophorus*, does have the apical sucker, but it has been recorded only from freshwater hosts of the southern hemisphere (23). Although the position of these larvae in the genus *Scyphophyllidium* seems most probable, it is felt that the diagnostic characteristics are not sufficiently well marked to attempt classification beyond *Phyllobothrium* sp. It should be noted that *Scyphophyllidium giganteum* (van Beneden, 1858) has been found in the thorny skate *Raia radiata* (=*Raja scabrata*) in the same waters as the present material (5).

Tetraphyllidea, plerocercoid

Host: *Limanda ferruginea.*
Location: Intestine, encysted in wall.
Locality: Entry Island, Magdalen Islands.

Two small larvae were removed from their site of encystment, close to the junction of the pyloric caeca. The coiled plerocercoid was 3.1 mm. long by 0.20 mm. wide. The scolex was rounded, 0.30 mm. in diameter, a slight constriction occurred between the scolex and the body.

Order SPATHEBOTHRIDIDEA
Family DIPLOCOTYLIDAE Wardle and McLeod, 1952
Genus *Diplocotyle* Krabbe, 1874

Diplocotyle olrikii Krabbe, 1874

Host: *H. platessoides,*
L. ferruginea,
Pseudopleuronectes americanus.
Location: Intestine of all three hosts.
Pyloric caeca of *L. ferruginea.*
Locality: *H. platessoides* and *L. ferruginea* in St. Mary's Bay, Nova Scotia.
P. americanus in St. Mary's Bay and Passamaquoddy Bay, New Brunswick.

This parasite was not found in any flatfish taken further north than Nova Scotia, and the infection was low in the parasitized fish. The yellowtail flounder carried only one tapeworm, and only 3% of the fish were infected. The winter flounder (*P. americanus*) was never parasitized by more than two tapeworms; the incidence was 13%. The four plaice examined from St. Mary's Bay carried only one or two specimens per fish.

D. olrikii has been found previously in *P. americanus*, Heller found 17 specimens in one fish. Unfortunately only three hosts were examined so no true incidence figure was recorded (5).

The size of the present material was variable, from 5 to 25 mm. in length by 0.30 to 0.80 mm. in width. The diameter of the round scolex was 0.50 to 0.70 mm., with the spherical holdfasts forming two separate units. This parasite is predominantly found in fish belonging to the genus *Salmo*; and it has

been suggested that the flatfish are abnormal hosts (21). This would in part account for the difference found in the pleuronectids recorded here and those of salmonoid hosts recorded elsewhere.

Order PSEUDOPHYLLIDEA
Family PTYCHOBOTHRIIDAE
Genus *Clestobothrium* Rudolphi, 1808

Clestobothrium crassiceps Rudolphi, 1808
Host: *Hippoglossus hippoglossus.*
Location: *Pyloric caeca.*
Locality: East Point, Anticosti Island.

There is only one previous record, also from the halibut (10), of this parasite in a pleuronectid anywhere in the world. The two cestodes were twisted together in one arm of the halibut's caeca. *C. crassiceps* is a common parasite of fish of the genus *Merluccius* (hakes) in most parts of the world. This unusual record may therefore be due to the ingestion of a hake by the halibut, with the consequent release of the tapeworm into the caeca. The specimens measured from 20 to 30 mm. in length, and neither carried mature segments.

Family BOTHRIOCEPHALLIDAE
Genus *Bothriocephalus* Rudolphi, 1808

Bothriocephalus scorpii Müller, 1776
Host: *Limanda ferruginea,*
Liopsetta putnami,
Scophthalmus aquosus.
Location: Caeca and intestine of *L. ferruginea* and the smooth flounder (*S. aquosus*).
Stomach, caeca, and intestine of the sand flounder (*Liopsetta putnami*).
Locality: Magdalen Islands; Grand River; St. Mary's Bay, Nova Scotia; Passamaquoddy Bay, New Brunswick.

All the sand flounders carried *B. scorpii*, in numbers ranging from one to six. The caecal arms were often blocked by the twisted and knotted cestodes. In some cases the worms would lie with their scoleces in the stomach of the host and their convoluted bodies passing through the intestinal caeca into the small intestine. There were from one to three *B. scorpii* present in the 14 specimens of *L. ferruginea* infected. The smooth flounder was more heavily infected, 30% carried from two to five tapeworms. *B. scorpii* is a cosmopolitan parasite and has been recovered from 21 species of Heterosomata in all oceans of the world.

Bothriocephalus claviceps (Goeze, 1782)
Host: *Pseudopleuronectes americanus.*
Location: Intestine.
Locality: Grand River.

A small winter flounder taken by a beach seine contained three cestodes measuring from 60 to 100 mm. in length and 1.5 to 2.2 mm. in width. The small holdfast, in the living specimen, was capable of rapidly changing shape. It elongated with the natural extension of the tapeworm, and became globular with the contraction of the cestode's strobila. The presence of the non-operculated eggs was useful in the diagnosis of this species. This parasite has been recorded previously from both *P. americanus* and *L. ferruginea* of the Woods Hole region (10).

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ON PRENATAL INFECTION AND THE MIGRATION OF TOXOCARA CANIS WERNER, 1782 IN DOGS[1]

GLORIA A. WEBSTER

Abstract

A definite age–sex–migration complex has been determined during studies on the migratory behavior of *Toxocara canis* Werner, 1782 in dogs. It has also been established that intrauterine infection occurs as a result of the reactivation of larvae in the somatic tissues of the bitch during gestation.

Although the importance of intrauterine infection in the epizoology of *Toxocara canis* has long been recognized, the method of infection and the factors necessary or responsible for it have not previously been established. Some workers (1, 7, 10) showed that prenatal infection could only take place when viable ova were ingested by the bitch during gestation. Courmelles (3) found, however, that female dogs clinically free from adult *Toxocara* produced infected litters. Taskin (in 3) considered there was a relationship between the life history of the parasite and prenatal infection. Yutuc (18) found that bitches free from intestinal infection and relatively isolated from any contact with helminth ova during gestation still produced litters infected with *T. canis* and *Ancylostoma caninum*. This led Yutuc to postulate that: ". . . the infection of the bitches which contributed to the parasitism of the pups had been acquired prior to the initiation of the gestation period, *a pregestation* infection." He considered that the larvae, immobilized in the somatic tissues of the host, became activated during pregnancy due to the lowered resistance of the host and the "debilitating effects of pregnancy," and travelled via the maternal circulatory system to the foetal tissue. My study has confirmed Yutuc's hypothesis that larvae in the somatic tissues serve as a source of intrauterine infection.

Previous work has elucidated the migration pattern and biology of *T. canis* and shown their importance in the establishment of prenatal infection. My study has clarified certain aspects of the migration which seem to have a direct bearing on this type of infection.

Stewart (14) and Ransom and Foster (9) were the first to demonstrate the similarity of the migration of *T. canis* larvae through the lungs and liver to that of *Ascaris lumbricoides*. The life history and migration were further studied by Yokogawa (17), Sprent (11, 13), and Wright (16). And the somatic migration to the muscles, central nervous system, kidneys, and other organs has been investigated by Ransom and Cram (8), Fülleborn (5, 6), Wright (16), and Sprent (11, 13).

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Methods

Eggs, obtained from the uterus of mature, female *T. canis* or from the faeces of infected dogs, were washed in tap water, centrifuged, and cultured in a 2% formalin solution at 25° C. for 1 month.

Dogs used in the migration study were given an anthelminthic on being brought into the laboratory and again 3 weeks later. Three or four dogs were kept in a pen until they were experimentally infected when they were removed to individual cages.

Dosages of from 1000 to 3000 infective eggs were force fed or fed mixed with a little milk. Twenty-one days after the initial infection a second quantity of eggs was administered, a third on the 28th day, a fourth on the 31st, and so on down to the last dose administered within four hours of the killing of the animal. Infections at planned intervals not only made it possible to collect from a single host larvae in various stages of development but also assured their presence in all locations. In order to determine the time required by larvae to reach certain sites, a single dose of 3000 to 5000 eggs was given.

After they were killed, each experimental animal was examined carefully, the liver, lungs, portions of muscle tissue, brain, kidneys, and intestine removed and digested with peptic digest solution in a Baermann apparatus. The larvae were collected and mounted for study.

In order to ensure absolute freedom from contact with *T. canis* eggs during pregnancy, the bitch used in the prenatal infection experiment was kept in a specially designed cage. The cage (Fig. 1) (58 in. by 33½ in. by 36 in.) was constructed of plywood with a sheet metal floor sloping towards a central small drainage hole. The lid (used only in placing the bitch in the cage) was fastened tightly. A small, hinged door served as an opening to permit feeding and cleaning. Filtered air was circulated in the box by means of a compressor.

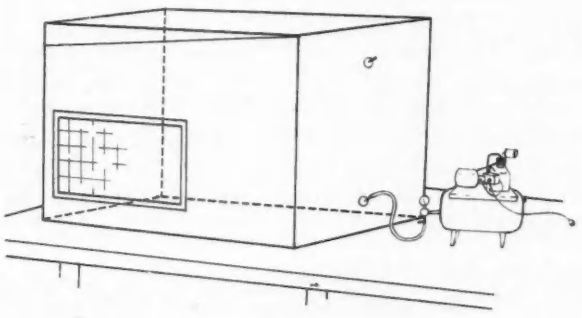

FIG. 1. Filtered-air cage.

Results

Migration

The basic migration pattern has already been described by several authors. The results of my own migration studies are included briefly as they form a basis on which may be studied the specific influence of host age and sex on the general migration pattern.

Most of the eggs hatch in the duodenum within 2 to 4 hours of ingestion and the liberated second-stage larvae penetrate the intestinal wall. While most of the larvae enter the lymph vessels, a few have been observed in the mesenteric blood capillaries. From the lymph nodes the larvae enter the venous capillaries and thus reach the portal circulation.

Within 24 hours some larvae have reached the liver and their number there continues to increase during the next 48 hours. During this phase there is no molt and only slight growth (0.41 to 0.49 mm.). Although no larvae were collected from the hepatic vein or vena cava, they have been collected from the heart and pulmonary artery 72 hours after infection, and it is assumed that this is the normal path of the larvae from the liver to the lungs. The number of larvae in the lungs reaches a peak between the 3rd and 5th day after which the number gradually decreases.

There are two possible routes for the larvae to follow from the lungs, either through the bronchioles to the trachea or via the pulmonary vein to the heart and circulatory system. Those which reach the mouth via the trachea are swallowed and reach the intestine via the oesophagus and stomach. Those which migrate to the heart and circulatory system are distributed to the somatic tissues.

As it was difficult to collect larvae from the trachea and oesophagus and as intestinal infections were not readily established in dogs over six months of age, the following experiment was carried out.

Six dogs (17 days, 8 and 12 weeks, 6 months, 1 and 2 years of age, respectively) were each fed 2000 embryonated *T. canis* eggs. The 17-day-old dog was killed and examined 11 days later. Many larvae were collected from the lungs, trachea, oesophagus, and stomach; none was found in the muscle tissue, two in the kidney, and eight in the brain. In the 8- and 12-week-old dogs examined 7 and 9 days, respectively, after infection, there were larvae in the lungs, oesphagus, and stomach; in both animals, larvae, although not numerous, were found in the muscle tissue and kidneys. The 6-month-old dog had larvae in the lungs, none in the trachea or oesophagus, a few in the stomach and intestine, and several hundred in the somatic tissues. Both older dogs had a large number of larvae in the somatic tissues; 13 larvae were recovered from the stomach of one, only two from the other. It would appear, therefore, that tracheal migration predominates in young dogs, somatic in older ones.

In young dogs there is a considerable growth of larvae in the lungs, where they measure 0.8 to 0.95 mm., or almost double the size of those in the liver. The second molt occurs in either the lungs, trachea, or oesophagus. By the 10th day the larvae have reached the stomach where they remain for several

days and where the third molt occurs; the larvae then measure between 1.0 and 1.5 mm. Fourth-stage larvae are present in the duodenum about 13 days after infection; molting fourth-stage larvae have been found between the 19th and 27th days. In experimentally infected dogs, ova begin to appear in the faeces between the 4th and 5th week.

In the lungs of older dogs larvae destined for a somatic existence neither molt nor develop beyond the second stage. The many hundreds of larvae examined from the somatic tissues of experimental dogs have all been in the second stage; these larvae survive in the tissues for at least six months.

It was noted that more larvae seemed to be recovered from the muscle tissues of female dogs than from male. To check this observation, one female and one male (10 months old) were each given three doses of 1000 embryonated eggs at different intervals. The dogs were killed on successive days and the same weight of muscle tissue taken from each dog was digested. Thirty per cent more larvae were recovered from the female than from the male.

Prenatal Infection

Two border collies, of unknown history, were brought into the laboratory 1 month before their litters were due. As they had been kept in pens with eight to 10 other dogs it was probable that they had been exposed to *T. canis* eggs. Faecal examinations were made twice weekly. While no ova were found during the month preceding parturition, ova were recovered from the faeces of both dogs 3 weeks after parturition. After anthelminthic treatment one dog passed an adult female and an adult male worm and the other dog, three mature female worms. It was impossible to determine if the dogs became infected during pregnancy. The pups in both litters were all infected.

A Scottish terrier belonging to the author was infected, when a pup, with *T. canis;* intermittent faecal examinations over a 2-year period failed to reveal the presence of adult worms after she was 9 months' old. This was a house dog and her outside contacts before and after breeding were limited to daily walks on a lead. Faecal examinations during pregnancy were negative. She had a litter of seven pups on September 2, 1955. In one pup, examined 24 hours after birth, 12 larvae (0.52 to 0.76 mm.) were collected from the lungs. (The remainder of the litter was reared for use in other experiments.) When the pups were 30 days old, eggs were being passed in the faeces of them all. The bitch, as already noted, was free from adult *T. canis* before and during pregnancy; 2 weeks after parturition eggs were recovered from her faeces and treatment resulted in the passing of two mature female and of one male worm.

Two females of this litter, kept in pens at the laboratory, have shown no indication of any intestinal infection since they were 7 months old. One of these females was bred on coming into season at 13 months of age. Although she was kept in a separate pen she was not completely isolated from contact with *T. canis* eggs as there were other dogs in the same room. One of her litter, examined 1 week after birth, contained three third-stage larvae in the stomach, and six fourth-stage larvae in the duodenum.

Immediately after breeding in December, 1957, the other bitch was removed to the specially designed cage and confined in it during pregnancy. This precluded the possibility of infection during the gestation period. Periodic faecal examinations were negative. Of the three pups born on February 24, 1958, one was killed 20 hours after birth, and eight third-stage larvae recovered from the lungs; all other tissues were negative. The two remaining pups were killed the following morning and 16 third-stage larvae recovered from the lungs of one and 12 from the lungs of the other; no other larvae were recovered.

Discussion

The study of the migratory behavior of *T. canis* in the dog has shown that the age of the host plays an important part in determining the ultimate fate of the larvae. It has shown, specifically, that the tracheal type of migration predominates in dogs under three months of age, and the somatic in those over six months old. The causes governing the two types of migration may be of a physiological nature connected with the age of the host.

The observation that more larvae migrate to the somatic tissues of female than of male dogs combined with the age–migration pattern can be correlated with Ehrenford's (4) findings. In a survey of 1324 dogs Ehrenford found 32.8% of the males but only 9.4% of the females infected with adult *T. canis*. Further analysis showed that, although puppies of both sexes had a high incidence of infection, the incidence in male dogs was "significantly higher" than in females. Ehrenford also found that male dogs showed no evidence of immunity up to 36 months of age, whereas females exhibited "a marked and increasing immunity from 6 to 36 months of age".

Intrauterine infection can be established either by the ingestion of infective ova during the gestation period or, through the reactivation of the second-stage larvae encapsulated in the somatic tissues. The age–sex–migration complex seems to be directly connected with the latter method of prenatal infection. That more larvae migrate to the somatic tissues of female than of male dogs and that, when the dogs are beyond six months of age, there is a marked tendency towards a somatic rather than a tracheal migration are important facts in that they ensure the maintenance of a high level of larvae in the tissues: the habits of dogs make frequent contact with *T. canis* eggs unavoidable.

In prenatal studies with *Trichinella spiralis* Augustine (2) concluded that in order to ensure the success of prenatal infection the larvae must meet two requirements. First, they must be active tissue penetrators and, secondly, they must have the ability to remain alive in the tissues of the host for extended periods of time. Second-stage larvae of *T. canis* fulfill both these requirements. In his study on the life history of *T. cati*, Sprent (13) reports that prenatal infection does not occur. The only point of deviation in the life histories of *T. canis* and *T. cati* which might impede prenatal infection in the latter is Sprent's (12) finding of third-stage larvae in the somatic tissues.

The mechanism by which encapsulated *T. canis* larvae are released from the somatic tissues is unknown, but it is thought that it may be connected with the hormone changes which accompany pregnancy. Taylor (15), in a symposium on the factors influencing host–parasite relationships, cited the case of *Strongyloides westeri*, which is common in foals. He observed that no matter how carefully the pregnant mares and foaling boxes were washed and disinfected, the foals were still infected. A few days before parturition, *Strongyloides* eggs were collected from the faeces of the mare; this was correlated with the beginning of activity in the mammary gland. Professor Wetzel (personal communication with Taylor) felt there was little doubt "that the oviposition of female worms that were presumably lying dormant in the mare, was activated by the hormones that appeared in the circulation of the mare at that time."

My study has once more indicated the complex relationships which can exist between a host and its parasite, and the importance of the life history and biology of an organism in determining its epizoology.

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PARASITES OF SOUTH PACIFIC FISHES

III. TREMATODES FROM THE SOLOMONS AND NEW HEBRIDES, WITH A DESCRIPTION OF DAITREOSOMA PARVA N. SP. (MONOGENEA) FROM GUADALCANAL[1]

MARSHALL LAIRD[2]

Abstract

Daitreosoma parva n.sp. (Monogenea: Dactylogyridae) was discovered in gill smears from four of five glassies, *Ambassis miops* Günther (Centropomidae), from fresh-water pools on Guadalcanal, British Solomon Islands Protectorate. Only two species of *Daitreosoma* were known previously, both of them parasitizing *Therapon* spp. (Theraponidae) from the Thomson River, Queensland. *D. parva* n. sp., measuring from 133 to 145 μ by from 38 to 41 μ (av., 139 by 39 μ), is only about one-quarter the length and width of its congeners. It also differs from these in details of the haptorial structures and genitalia, notably in that the testis is ventral to the ovary. An encysted metacercaria of *Stephanostomum* sp. (Digenea: Acanthocolpidae) is also recorded from the gills of *A. miops*. One of 12 mullets, *Mugil oligolepis* Bleeker (Mugilidae), from a tidal stream on the island of Aneityum, New Hebrides, had an example of an undetermined species of *Microcotyle* (Monogenea: Microcotylidae) on its gills.

Methods and Material

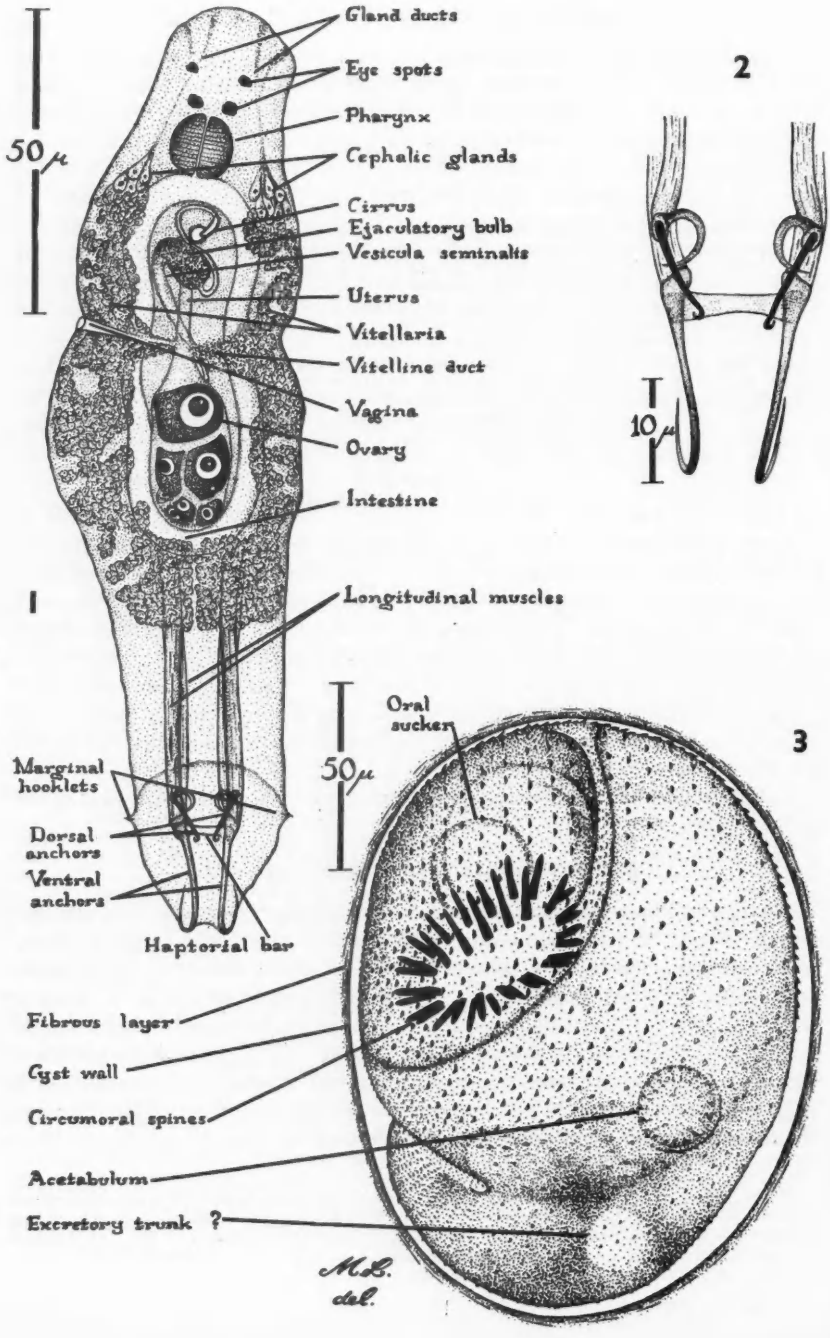
These trematodes were found in gill smears from fish immobilized with rotenone (5%). Their hosts, a mullet, *Mugil oligolepis* Bleeker (Mugilidae), and a glassy, *Ambassis miops* Günther (Centropomidae), commonly frequent Indo-Pacific estuaries. The mullets were taken from a flowing tidal stream on Aneityum, New Hebrides, while the glassies and other fish (4) had become isolated in fresh-water ponds near the mouth of a drying-up river on Guadalcanal, British Solomon Islands Protectorate. *A. miops*, which ranges from India to central Polynesia, often enters rivers from brackish waters and the sea (8).

Gill impression smears were prepared, fixed, and stained as described in the earlier papers of this series (4, 5).

Daitreosoma Johnston and Tiegs, 1922

The family Dactylogyridae Bychowsky was briefly characterized in the preceding paper (5). Its subfamily Tetraonchinae Monticelli embraces those species in which the body is devoid of scales or spines while the haptor lacks squamodiscs but bears two pairs of anchors and two to 16 marginal hooklets (7). *Daitreosoma* is distinguished from the many other genera of this family by its simple transverse haptorial bar, its bifurcate intestine with confluent caeca, the presence of only two marginal hooklets and the occurrence of a vagina which opens on the left margin at a constriction marking the junction of the anterior and middle thirds of the body (3, 7).

[1]Manuscript received March 3, 1958.
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Macdonald College P.O., Que., Canada. The material was collected during a research project initiated by the Royal New Zealand Air Force and supported by a grant from the New Zealand Department of Scientific and Industrial Research.
[2]Institute of Parasitology, McGill University.


Gland ducts
Eye spots
Pharynx
Cephalic glands
Cirrus
Ejaculatory bulb
Vesicula seminalis
Uterus
Vitellaria
Vitelline duct
Vagina
Ovary
Intestine
Longitudinal muscles
50 μ
1
2
10 μ
Oral sucker
50 μ
3
Marginal hooklets
Dorsal anchors
Ventral anchors
Haptorial bar
Fibrous layer
Cyst wall
Circumoral spines
Acetabulum
Excretory trunk ?
M.L. del.

Only two species of *Daitreosoma* were known hitherto, both of them being gill parasites. *D. constrictum* Johnston and Tiegs, 1922, from *Therapon carbo* Ogilby and McCulloch (Theraponidae), and *D. bancrofti* Johnston and Tiegs, 1922, from *T. hilli* Castelnau, were both described from the Thomson River at Long-reach, central Queensland, Australia (3).

Daitreosoma parva n. sp.

(Figs. 1 and 2)

Host: *Ambassis miops* Günther (immature and adult, 68 to 85 mm.).
Locality: Mouth of Poha River, Guadalcanal, British Solomon Islands Protectorate.
Date: September 11, 1953.

Description based on five individuals derived from four of the five examples of the host collected.

Body elongate when fixed and stained (Fig. 1), 7:2. Over-all measurements, 133 to 145μ (av., 139μ) by 38 to 41μ (av., 39μ). Haptor no wider than posterior part of body proper. The two marginal hooklets extending from tiny lateral papillae at junction of haptor and body proper. Ventral anchors 18.5 to 20μ (av., 19μ) from base to top of curve of hook, the thin point recurved for one-third to two-fifths of the length of the shank (Figs. 1 and 2). Dorsal anchors (Fig. 2) shaped like crochet hooks, 7 to 9μ (av., 8μ) long. Strap-shaped haptorial bar joining the ventral anchors basally at the level of the hooks of the dorsal anchors, 11.5 to 13μ (av., 12.5μ) long, and C-shaped accessory pieces between the bases of the dorsal and ventral anchors of each side (Fig. 2). Longitudinal muscles well developed (Fig. 1), originating in the middle third of the body and ending at the bases of the anchors.

Head 21 to 24μ (av., 22μ) wide basally, location of mouth and course of esophagus not determined. Cephalic glands laterally positioned, posterior to the mid-region of the pharynx. Gland ducts proceeding anteriorly, terminating in swollen "head organs". While only two pairs of these can be distinguished with certainty, the staining reaction is poor and more could be present. Sproston (7, p. 215) used the presence of but two pairs of "head organs" as a key character for *Daitreosoma*. This is an error. Her generic diagnosis (7, p. 234) states that three pairs occur, but Johnston and Tiegs (3), who showed that *D. constrictum* has three pairs, clearly illustrated four pairs in *D. bancrofti* (their Pl. XIII, Fig. 22). Posterior pair of eye spots slightly in advance of the pharynx, the anterior pair smaller and more widely separated (Fig. 1). Pharynx prominent, 10 to 14μ (av., 12μ) long and 9.5 to 11μ (av., 10μ) wide, giving access to the continuous intestine, the figure-of-eight shape of which is determined by the constriction near the junction of the

FIGS. 1–3. Figures prepared with the aid of a Zeiss-Winkel drawing apparatus, from preparations stained with iron haematoxylin.
FIG. 1. *Daitreosoma parva* n. sp. Whole animal, dorsal view. ×900
FIG. 2. *Daitreosoma parva* n. sp. Haptorial structures, dorsal view. ×1550
FIG. 3. *Stephanostomum* sp. Encysted metacercaria. ×550

anterior and middle thirds of the body. Ventrolaterally, in the region of the constriction, are deeply staining cells (not illustrated) comparable with those seen in *D. constrictum* by Johnston and Tiegs (3), who suggested that they might be digestive glands.

Testis oval and directly ventral to the ovary, the vas deferens proceeding forward and expanding to form a vesicula seminalis behind and to the left of the male pore. Ejaculatory bulb prominent, a narrow duct leading to the tubular cirrus.

Ovary longitudinal, containing six or seven very conspicuous and deeply staining oocytes which become progressively larger anteriorly. Over-all measurements, 24 to 28 μ (av., 25μ) by 13 to 19μ (av., 16μ). Vagina leading into the oviduct at the level of the vitelline duct, its point of union with which remains undetermined. Uterus a simple tube, which cannot be traced further forward than the ejaculatory bulb in the specimens at hand. Vitellaria extending from the level of the cephalic glands to near the junction of the middle and posterior thirds of the body as in the two species already known (3).

In both *D. constrictum* and *D. bancrofti* the testis is posterior to the ovary, not ventral to it. The former species has dorsal hooks very like those of the *Ambassis* parasite, but its ventral hooks, as illustrated by Johnston and Tiegs (their Pl. XII, Fig. 18), are relatively broad and have their tips bent more or less at right angles instead of being sharply recurved. Furthermore, the accessory pieces linking the dorsal and ventral anchors of each side are Y-shaped, not C-shaped, in *D. constrictum*. This species, measuring about 450 by 160μ, is very much larger than the one under discussion. *D. bancrofti*, the haptorial structures of which have not been described, is larger still (about 560 by 190μ). The Guadalcanal species is thus regarded as new, and by virtue of its small size it is designated *Daitreosoma parva* n. sp.

The type slide has been deposited in the Helminthological Collection of the United States National Museum, and a paratype is lodged at the Dominion Museum, Wellington, New Zealand.

Stephanostomum sp.

(Fig. 3)

Field data as for the preceding species, the host, an 85 mm. *Ambassis*, being parasitized by *Daitreosoma* as well.

The one metacercaria located is enclosed in an oval, thin-walled cyst (160 by 126μ) surrounded by a fibrous layer of host tissue (Fig. 3). Owing to the manner in which the worm is folded upon itself, a full length reconstruction is not practicable. Its most prominent feature is a crown of 34 deeply staining spines arranged alternately in two unbroken concentric rows, the diameter of the outer one being about 40μ. The spines are thickest basally and are bluntly pointed, the longest of them being in the inner row. A sucker, identified as the acetabulum, is apparent towards the opposite side of the rolled-up body. It is slightly smaller than the oral sucker, which is partly obscured by

the circlets of spines. Circular clear zones (Fig. 3) are interpreted as portions of the excretory trunks seen in sectional view. Rows of tiny spines are evident over the entire cuticle.

On first arrival at the ponds, herons (*Egretta sacra sacra* (Gmelin)) were feeding there. It was thus thought possible that the cyst was that of an avian parasite belonging to the Echinostomatidae or Heterophyidae, for members of both of these families are known from herons (2). The subfamilies and relevant genera characterized by a double circlet of oral spines are the Echinostomatinae (*Echinoparyphium* Dietz) and Centrocestinae (*Ascocotyle* Looss). However, the collar of species of the former genus bears prominent corner spines, and the acetabulum is well developed in *Ascocotyle* as in the Centrocestinae in general. The relatively small size of the acetabulum, the form of the circlets of spines and the cuticular spination identify the organism as belonging to the family Acanthocolpidae. As the sequence of oral spines is not interrupted ventrally and the spines of the two circlets alternate, it is referred to *Stephanostomum* Looss (2, 10). The general form of the cyst, with its thin, membranous inner wall and a thick outer wall laid down by the host, agrees with descriptions of encysted metacercariae of this genus (6, 9). Identification beyond the generic level is impracticable.

Microcotyle sp.

Host: *Mugil oligolepis* Bleeker (young example, 62 mm.).
Locality: Anelgauhat, Aneityum, New Hebrides.
Date: March 5, 1953.

Adults of the genus *Microcotyle* (Monogenea: Microcotylidae) have an equal number of discocotylid clamps of uniform size on either side of a haptor lacking anchors and terminating posteriorly at the posterior limit of the body proper (7). A worm of this genus was recognized during phase-contrast examinations of fresh material, but further specimens could not be found when cover slip smears prepared at the same time were stained and searched.

Host Relationships

Ambassis is a new host for trematodes, but three species of *Microcotyle* have been described from *Mugil cephalus* L. (1, 7), a fact which makes it doubly unfortunate that permanent preparations of the species from *M. oligolepis* were not obtained. It would be instructive to compare the South Pacific species with those already known from the same host genus in the Mediterranean, Japan and Baja California, Mexico.

Daitreosoma parva n. sp. resembles its congeners in its fresh-water habit, although in view of the wide tolerances of the host it might prove to occur in saline waters as well. Monogenea are characteristically restricted in their choice of hosts, and the fact that *D. parva* n. sp. was found on the gills of four out of five examples of *Ambassis miops* although it was absent from 24 fish of seven other species in the same ponds (4) points to marked host specificity.

Fish are the definitive, as well as the second intermediate hosts, of *Stephanostomum*. No light can be shed on likely definitive hosts for the Guadalcanal species, but aquatic snails are the first intermediate hosts for this genus and three species of gastropods were abundant in the ponds concerned: *Melania funiculus* Quoy and Gaimard, *M. verrucosa* Hinds, and *Navicella suborbicularis* Sowerby.

Acknowledgments

Thanks are due to my wife for her help in the field, and to Dr. Paul C. Beaver for advice on the systematic position of the encysted metacercaria from *Ambassis miops*.

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STUDIES ON STRONGYLOIDES OF PRIMATES

III. OBSERVATIONS ON THE FREE-LIVING GENERATIONS OF S. FÜLLEBORNI[1]

PREMVATI[2]

Abstract

There is only a single generation of free-living adults in the life cycle of *S. fülleborni*. The free-living mature female lays a batch of eggs after each mating, thus increasing the number of infective larvae. Unfertilized females of the exogenic generation are unable to lay viable eggs.

Strongyloides fülleborni von Linstow (7) under optimum conditions shows a predominantly indirect or heterogonic development, that is, in the external medium adult males and females develop and give rise to infective larvae only (9). Mature, free-living adults are produced after 48 hours' incubation at 25° C., and the females begin laying embryonated eggs 4 hours later. The infective larvae take 2 days to develop from these eggs. Thus, from the 5th day of the commencement of incubation, infective larvae with a few free-living adults can be obtained by baermanning the cultures, while after 6 to 7 days, the cultures show only infective larvae.

In order to ascertain if there is a second generation of free-living adults, the experiments were begun with mature worms, baermanned from the cultures after 48 hours. The experiments were divided into two series: A, having both males and females; B, females only. The numbers were counted at the beginning of the experiments and each subsequent day. The cultures were made in three groups with a known number of worms in each:

Group I.—Sterilized cow faeces and charcoal (natural media).

Group II.—Tap water to which a small quantity of food from the same culture was added.

Group III.—Nutrient agar.

The experiments were repeated five times on different dates with 12 cultures of each group each time. The average numbers are recorded in Tables I, II, and III. The results obtained in the different cultures are as follows:

Group I.—After 1 day the cultures showed a large number of rhabditiform and preinfective larvae in both A and B. In examinations subsequent to the 2nd day, the numbers of rhabditiform, preinfective, and infective larvae in series A increased while in B only infective larvae and a very small number of rhabditiform larvae were seen. At the end of the 6th day the cultures contained no free-living adults and in no culture did their number increase during the entire experiment. After the 6th day, the number of infective larvae per female was four to five times more in series A than in B (Table I).

[1]Manuscript received January 10, 1958.
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Macdonald College P.O., Que., Canada, with financial assistance from the National Research Council of Canada.

[2]Margaret McWilliam Fellow (awarded by Canadian Federation of University Women). At present on leave from the Department of Zoology, University of Lucknow, Lucknow, India.

TABLE I

NATURAL MEDIA

Date	Females	Males	Time in days	Total no. of larvae obtained	Average no. of larvae per female
			A Series experiments		
8.5.57	24	24	6	4000	166.7
17.5.57	30	30	6	4800	160
25.5.57	12	12	5	1896	158
10.6.57	20	10	5	2750	137.5
15.6.57	2	2	5	250	125
			B Series experiments		
8.5.57	24	–	6	1400	58.3
17.5.57	20	–	5	1000	50
25.5.57	12	–	5	450	37.5
10.6.57	20	–	4	660	33
15.6.57	2	–	4	70	35

Group II.—In liquid media the life of the free-living adults is short and as they die in 3 days the larvae were counted after this period. Some of the rhabditiform and filariform larvae also died but unless they had totally disintegrated they were included in the count. Table II shows the number of infective larvae per female to be much reduced.

TABLE II

TAP WATER

Date	Females	Males	Time in days	Total no. of larvae obtained	Average no. of larvae per female
			A Series experiments		
8.5.57	2	2	3	170	85
17.5.57	4	4	4	368	92
25.5.57	1	1	3	74	74
10.6.57	20	20	4	1400	70
15.6.57	12	12	3	850	70.8
			B Series experiments		
8.5.57	2	–	3	75	37.5
17.5.57	4	–	4	110	27.5
25.5.57	1	–	3	35	35
10.6.57	14	–	2	500	35.7
15.6.57	10	–	2½	380	38

Group III.—In order to facilitate microscopic examination and at the same time provide the correct consistency to keep the worms alive for a longer period, a very thin layer of nutrient agar was used. These cultures were examined hourly from 7.00 a.m. to 10.00 p.m. for 6 days, as long as the adults were alive.

In none of these cultures was a second generation of free-living adults observed; the number of males and females remained constant throughout the experiment. Rhabditiform larvae gave rise only to infective larvae. The total number of infective larvae obtained per female (Table III) increased to some extent as compared with those in Group II but not to the same extent as in natural media.

TABLE III

NUTRIENT AGAR

Date	Females	Males	Time in days	Total no. of larvae obtained	Average no. of larvae per female
			A Series experiments		
8.5.57	6	6	4	564	94
17.5.57	10	10	4	800	80
25.5.57	2	2	3	120	60
10.6.57	1	1	3	70	70
15.6.57	20	10	5	1700	85
			B Series experiments		
8.5.57	10	–	3	450	45
17.5.57	6	–	4	312	52
25.5.57	2	–	5	64	32
10.6.57	1	–	4	36	36
15.6.57	20	–	4	1100	55

Mature females when left without males lay the first batch of embryonated eggs 52 hours after the commencement of incubation. When examined under the microscope these females, after they had laid the first batch of eggs, are seen to have very few eggs in the uteri. About 10 to 12 hours later they are seen to lay these eggs—never more than 10 in number. Subsequent to this laying, the females are unable to lay any more fertilized eggs. The reverse is true of mature females maintained with males: their uteri are never empty and even after the death of the female embryonated eggs hatch within the body and are liberated upon the disintegration of the parent.

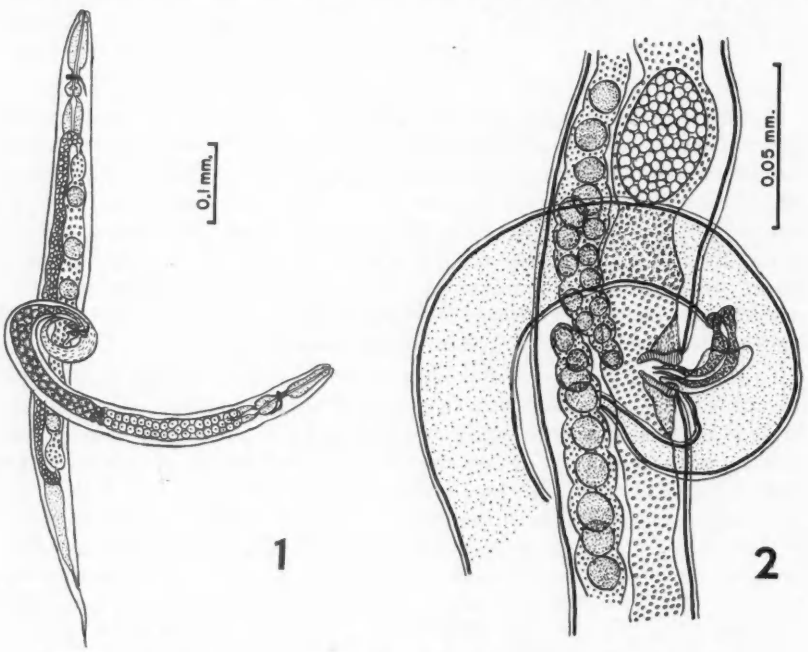
To find out if unaccompanied females are capable of laying viable eggs, immature females and males were baermanned after 24 to 28 hours' incubation. In most of the females the vulvar openings had not formed and in none had fertilization taken place. Females only were maintained in culture in six small dishes and males and females in another six dishes. After 2 days the unaccompanied females were observed to have very few eggs (five to eight) in their uteri. The eggs were in their early developmental stages and, not reaching the embryonation stage, did not hatch. When examined microscopically the females showed ova but no sperm in the receptacula seminalis. Moreover, the characteristic constriction below the vulva, which gives a waist-like appearance to the mature females, was missing (8). In the cultures containing males and females a number of rhabditiform and infective larvae

were seen. Microscopic examination of these females (which had the characteristically shaped vulva) revealed the presence of sperm in the receptacula seminalis.

The facts that, under optimum conditions, the eggs of the parasitic female invariably give rise to free-living adults, that the eggs of free-living adults give rise to filariform larvae, and that the number of filariform larvae produced by one pair is much greater than the number of eggs present in the female's uterus at any one time, led to further investigations. Continuous observation revealed that the free-living female copulates a number of times and thus continues egg-laying throughout her life. The mature female separated from the male lays no more than 40 to 60 embryonated eggs, whereas a female accompanied by a male lays as many as 180 eggs.

The free-living adults generally copulate after 30 hours' incubation; in one culture seven to eight pairs were seen in copula after 32 hours' incubation. The latter were examined both alive and fixed.

Mature males and females were baermanned from a culture maintained for 48 hours at 25° C. One adult female was placed in each of six solid watch glasses. Four to six hours later (that is, after 52 to 54 hours' incubation) each watch glass contained between 30 and 35 rhabditiform larvae. Each female was then placed on a separate micro-counting slide in fresh media and



FIGS. 1 and 2. Free-living adult female and male of *S. fülleborni* in copulation.
FIG. 1. Under low power magnification. FIG. 2. Under high power magnification.

a male (of the same lot) added to each slide. The slides were examined continuously under the dissecting binocular. Copulation took place within 10 to 15 minutes. The male, after several attempts to coil its posterior end around the female, catches it in the middle, coils its posterior end round the vulvar region, and inserts its spicules into the vagina. While at times the male coils its whole body three or four times around the female, usually its posterior half from the region of the vas deferens coils twice around during the full time of copulation (Figs. 1, 2). The anterior end remains free, relaxing and contracting. The female shows very slow movement during the process. Copulation takes from 3.24 minutes to 3.45 minutes, following which the male uncoils and relaxes for a second before moving actively away from the female. The female also remains still for a few seconds before becoming very active.

Following a second copulation the females were again separated from the males and 12 to 14 hours later began laying a second batch of embryonated eggs from which 20 to 25 rhabditiform larvae hatched. The same females were again allowed to mate with males of the same lot; after 20 minutes a third copulation took place in two of the six pairs, this time requiring 2.40 minutes in one pair and 2.24 minutes in the other. Although the remaining four pairs were examined continuously for 3 hours, mating did not take place. Twelve to sixteen hours after the third copulation, a third batch of from 20 to 25 rhabditiform larvae were produced by each female. In liquid media the life of the free-living adult is too short to allow of a fourth mating.

That females mate a number of times during their life was confirmed by another set of 12 experiments. Single pairs of mature females and males were placed on solid watch glasses with culture media. The number of filariform larvae obtained from these cultures was much greater (120 to 160) than the number obtained from females separated from the males after each mating. Even in liquid media the number of larvae increases if the pairs remain together.

The need for repeated mating was also studied morphologically. After 36 hours' incubation the receptacula seminalis of the female contains a large number of sperms and the uterus a small number of eggs in early developmental stages. The latter most probably pass to the uterus after fertilization. Twelve to 14 hours later the receptacula seminalis is obscured by the fully embryonated developing eggs. The females were re-examined after the first batch of eggs had been laid and the receptacula seminalis was clearly discernible and contained few sperms. It can, therefore, be concluded that the female is unable to store sufficient sperms for the ova budded off from the germinal epithelium of the ovary during its life time. Repeated mating is necessary for a continuous supply of fertilized eggs.

Discussion and Conclusions

Even under optimum conditions, in the life cycle of *S. fülleborni* there is only one generation of free-living adults. Rhabditiform larvae hatched from the eggs of the free-living females give rise to infective larvae. This agrees

with Leuckart (5) and Looss (6), who believed that the free-living adults of *S. stercoralis* produced only filariform larvae and that there was no second generation of free-living adults.

Beach (1, 2) showed experimentally that in *S. simiae* the average number of larvae produced in culture by each free-living female was 60, with a maximum of 80. Since after four days, from nine eggs developed into four males, four females, and one first generation filariform larva, he got 629 individuals, he considered that there is propagation in the free-living generation. Faust (3) also agrees with Beach and is of the opinion that *Strongyloides* may be propagated exclusively as free-living organisms. Kouri, Basnuevo, and Arenas (4) have recorded a step further in observing propagation in *S. stercoralis*, and that after numerous such generations the worms become entirely free-living, the females become parthenogenetic and no males are produced, the fecundity of the females being gradually lost until the cultures become sterile.

The present investigation has shown that a single pair, if left undisturbed, can produce as many as 180 infective larvae. An unaccompanied mature female lays no more than 40 to 60 eggs. Thus the greater number of infective larvae obtained from a particular number of free-living adults would depend mainly upon the repeated mating and not on their second generation. The free-living female lays eggs in batches following each mating. In liquid media, even although the life is short, as many as three copulations were observed in single couples, and the number of eggs laid after each mating was 30 to 35, 20 to 25, and 20 to 25, respectively. The copulation process and the time taken each time was also observed. The unfertilized females of the exogenic generations are unable to lay viable eggs.

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Contents